REGISTRATION REPORT

Part B

Section 9: Ecotoxicology

Detailed summary of the risk assessment

Product code: GLOB1310aH

Product name(s): Glosset Ace

Chemical active substances:

Aclonifen, 540 g/L
Flufenacet, 60 g/L

Central Zone

Zonal Rapporteur Member State: Poland

CORE ASSESSMENT

(authorization)

Applicant: Globachem NV

Submission date: December 2021

MS Finalisation date: 25/08/2022

After commenting: 14/12/2022

Version history

When	What
December 2021	Initial submission by the applicant for the approval of a new formulation
August 2022	First zRMS PL evaluation
December 2022	Corrections made by zRMS PL after commenting round

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9 Ecotoxicology (KCP 10)

9.1 Critical GAP and overall conclusions

Table 0-1: Table of critical GAPs

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Use-No. *	Member state(s)	Crop and/or situation (crop destination / purpose of crop)	F, Fn, Fpn G, Gn, Gpn or I **	Pests or Group of pests controlled (additionally: develop-mental stages of the pest or pest group)	**Application**				**Application rate**			PHI (days)	Remarks: e.g. g safener/ synergist per ha	**Conclusion**						
					Method / Kind	Timing / Growth stage of crop & season	Max. number a) per use b) per crop/ season	Min. interval between applications (days)	kg or L product/ha a) max. rate per appl. b) max. total rate per crop/season	g or kg as/ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min/max			Birds	Mammals	Aquatic organisms	Bees	Non-target arthro-	Soil organisms	Non-target plants
Zonal uses (field or outdoor uses, certain types of protected crops)																				
1-6	PL BE CZ DE HU IE RO SI SK	Winter cereals Winter Wheat (TRZAW) Winter Barley (HORVW) Winter Oat (AVESW) Winter Rye (SECCW) Winter Triticale (TTLWI) Winter Durum Wheat (TRZDW)	F	Annual weeds (BBBBB)	Normal downward spraying	BBCH 00-09 (Sep-Dec)	a) 1 b) 1	/	a) 1.5 L/ha b) 1.5 L/ha	a) 0.810 kg Aclonifen/ha + 0.090 kg Flufenacet/ha b) 0.810 kg Aclonifen/ha + 0.090 kg Flufenacet/ha	150-300	Not relevant, see application stage	/							
7-12	PL BE CZ DE HU IE RO SI SK	Winter cereals Winter Wheat (TRZAW) Winter Barley (HORVW) Winter Oat (AVESW) Winter Rye (SECCW)	F	Blackgrass (ALOMY)	Normal downward spraying	BBCH 00-09 (Sep-Dec)	a) 1 b) 1	/	a) 2 L/ha b) 2 L/ha	a) 1.08 kg Aclonifen/ha + 0.120 kg Flufenacet/ha b) 1.08 kg Aclonifen/ha + 0.120 kg Flufenacet/ha	200-300	Not relevant, see application stage	/							

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
		Winter Triticale (TTLWI) Winter Durum Wheat (TRZDW)																		
Interzonal uses (use as seed treatment, in greenhouses (or other closed places of plant production), as post-harvest treatment or for treatment of empty storage rooms)																				
Minor uses according to Article 51 (field uses)																				
Minor uses according to Article 51 (interzonal uses)																				

* Use number(s) in accordance with the list of all intended GAPs in Part B, Section 0 should be given in column 1

** F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application

Explanation for column 15 – 21 “Conclusion”

A	Acceptable, Safe use
R	Further refinement and/or risk mitigation measures required
C	To be confirmed by cMS
N	No safe use

Remarks table:

(1) Numeration necessary to allow references
(2) Use official codes/nomenclatures of EU
(3) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(4) F: professional field use, Fn: non-professional field use, Fpn: professional and non-professional field use, G: professional greenhouse use, Gn: non-professional greenhouse use, Gpn: professional and non-professional greenhouse use, I: indoor application
(5) Scientific names and EPPO-Codes of target pests/diseases/ weeds or when relevant the common names of the pest groups (e.g. biting and sucking insects, soil born insects, foliar fungi, weeds) and the developmental stages of the pests and pest groups at the moment of application must be named
(6) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench
Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(7) Growth stage at first and last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(8) The maximum number of application possible under practical conditions of use must be provided
(9) Minimum interval (in days) between applications of the same product.
(10) For specific uses other specifications might be possible, e.g.: g/m³ in case of fumigation of empty rooms. See also EPPO-Guideline PP 1/239 Dose expression for plant protection products
(11) The dimension (g, kg) must be clearly specified. (Maximum) dose of a.s. per treatment (usually g, kg or L product / ha).
(12) If water volume range depends on application equipments (e.g. ULVA or LVA) it should be mentioned under “application: method/kind”.
(13) PHI - minimum pre-harvest interval
(14) Remarks may include: Extent of use/economic importance/restrictions

9.1.1 Overall conclusions

9.1.1.1 9.1.1.1 9.2 Effects on birds (KCP 10.1.1)

9.1.1.2

9.1.1.3 Review comments:

The risk assessment to birds was performed in accordance with the recommendation of Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA (EFSA Journal 2009; 7(12):1438).

The results of the 'screening phase' acute dietary risk assessment and Tier-1 long term dietary risk assessment - Toxicity Exposure Ratios (TER_A and TER_{LT}) were calculated taking into account the EU agreed endpoints for most sensitive species for the active substance and using the EFSA Bird and Mammal risk assessment calculator for the higher predicted application rate than it is foreseen in GAP exceeding the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. Revealed that there is no potential of risk for birds resulting from acute and long-term exposure to active substance following use of GLOB1310aH in compliance with proposed GAP.

A quantitative drinking water risk assessment is not triggered for the proposed use pattern of GLOB1310aH according to EFSA/2009/1438 criteria and therefore the risk to birds via drinking water is acceptable.

Since the acceptability criterion of TER $\geq$ 5 is achieved for all active substances, an acceptable risk to earthworm-eating and fish-eating birds via secondary poisoning can be concluded for all intended uses.

No risk mitigation measures are required.

Conclusion

According to the performed risk assessment there is no potential of risk to birds resulting from exposure to active substance following use of Glosset Ace (GLOB1310aH) in compliance with proposed GAP.

9.3 Effects on terrestrial vertebrates other than birds (KCP 10.1.2), 9.4 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

The acute and long term risk to birds and mammals is acceptable when following the proposed GAP of GLOB130aH.

9.1.1.2 9.5 Effects on aquatic organisms (KCP 10.2)

The risk assessment of flufenacet is unsolved in the D1, D2 and D6; however, none of these scenarios is relevant in the Central Zone. Therefore, no mitigation measures or restrictions related to these scenarios are necessary. For the other scenarios a resume of mitigation measures in presented in the table below.

FOCUS scenario	Conclusions			
	1.5 L/ha	1.5 L/ha (VFSMOD)	2.0 L/ha	2.0 L/ha (VFSMOD)
D3 Ditch	5m nsb + 75% drn OR 10m nsb + 50% drn	-	5m nsb + 75% drn OR 10m nsb + 50% drn	-
D4 Pond	5m nsb	-	5m nsb	-
D4 Stream	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb	-	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn	-
D5 Pond	5m nsb	-	5m nsb	-
D5 Stream	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb	-	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn	-
R1 Pond	5m nsb	-	5m nsb	-
R1 Stream	10m vfs + 50% drn	10m vfs	20m vfs	10m vfs + 50% drn
R3 Stream	20m vfs	20m vfs	20m vfs + 50% drn	20m vfs + 50% drn
R4 Stream	10m vfs + 50% drn	10m vfs	20m vfs	10m vfs

If the combined risk assessment presented by Applicant is not accepted, the further risk assessment should be considered on the MS level.

9.1.1.3 Błąd! Nie można odnaleźć źródła odwołania.

The acute and long term risk to honey bees (adult and larvae) and to bumble bees is acceptable when following the proposed GAP of GLOB130aH.

9.1.1.4 9.7 Effects on arthropods other than bees (KCP 10.3.2)

The in- and off-field risks for the non-target arthropods *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Aleochara bilineata* and *Poecilus cupreus* are acceptable for the intended use in winter cereals as recommended in the GAP of GLOB1310aH.

9.1.1.5 9.8 Effects on non-target soil meso- and macrofauna (KCP 10.4), 9.9 Effects on soil microbial activity (KCP 10.5)

All TER values exceed their respective triggers, indicating that GLOB1310aH poses a low acute and chronic risk to earthworms and low long-term risks to other soil macro- and mesofauna when applied according to the proposed uses.

As the PEC_{soil} of Aclonifen, Flufenacet, its metabolites and the formulation are all lower than the concentration at which no significant effects are detected, it can be concluded that the risk GLOB1310aH to soil micro-organisms is acceptable in accordance with all intended uses.

9.1.1.6 9.10 Effects on non-target terrestrial plants (KCP 10.6)

The risk to non-target terrestrial plants is considered acceptable when GLOB1310aH is used according to the proposed GAP.

9.1.1.7 9.11 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

Tests on other non-target species are not required.

9.1.2 Grouping of intended uses for risk assessment

The following table documents the grouping of the intended uses to support application of the risk envelope approach (according to SANCO/11244/2011).

Table 0-2: Critical use pattern of GLOB1310aH grouped according to application rate

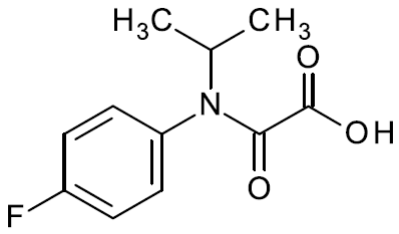
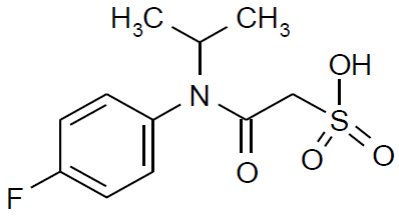
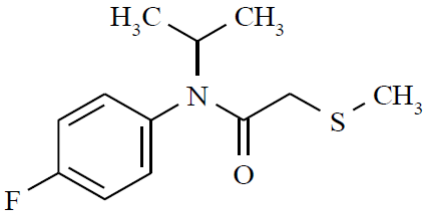
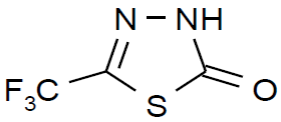
Grouping according to application rate			
Group	**Intended uses**	**relevant use parameters for grouping**	**relevant parameter or value for sorting**
1	Uses 1 to 6 Winter cereals	Application rate	1 x 1.5 L/ha (1 x 810 g a.s./ha aclonifen, 1 x 90 g a.s./ha flufenacet)
2	Uses 7 to 12 Winter cereals	Application rate	1 x 2.0 L/ha (1 x 1080 g a.s./ha aclonifen, 1 x 120 g a.s./ha flufenacet)

9.1.3 Consideration of metabolites

A list of metabolites found in environmental compartments is provided below. The need for conducting a metabolite-specific risk assessment in the context of the evaluation of GLOB1310aH is indicated in the table.

For Aclonifen no major metabolites are identified.

Table 0-3 Metabolites of Flufenacet

Metabolite	Chemical structure	Molar mass	Maximum occurrence in compartments	Risk assessment required?
FOE oxalate (M1)		225.2 g/mol	Soil: 15.6% Water/Sediment: 5.4%	Aquatic: Yes Soil: Yes
FOE sulfonic acid (M2)		275.3 g/mol	Soil: 26.3% Water/Sediment: 3.2%	Aquatic: Yes Soil: Yes
FOE methylsulfide (M5)		241.3 g/mol	Soil: water metabolite Water: 82%	Aquatic: No Soil: No
FOE-thiadone (Thiadone, M9)		170.1 g/mol	Soil: 3.9% Water/Sediment: 11.4% (8% in water, 3.4% sediment)	Aquatic: Yes Soil: No

9.2 Effects on birds (KCP 10.1.1)

9.2.1 Toxicity data

Avian toxicity studies have been carried out with Aclonifen. Full details of these studies are provided in the respective EU DAR and related documents. Effects on birds of GLOB1310aHwere not evaluated as part of the EU assessment of Aclonifen. However, the provision of further data on the GLOB1310aH is not considered essential, because active substance data on toxicity to birds are used.

Birds are typically exposed to dry residues on their food items following the dilution and spraying of the formulated product. During these processes, much of the formulation constituents are likely to be lost by volatilisation. Since oral exposure is the main route of exposure, toxicity data for the active substances are therefore used in preference to data from tests with the formulated material. On this basis, the risk to birds from the proposed use of GLOB1310aH will be assessed using data on Aclonifen. Therefore, all relevant data were assessed in the EU review. Risk assessments for GLOB1310aH with the proposed use pattern are provided here and are considered adequate.

The EU agreed endpoints for the avian toxicity studies are summarised in table 9.2-1 below.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 0-1: Endpoints and effect values relevant for the risk assessment for birds

Species	Substance	Exposure System	Results	Reference
Bobwhite quail	Aclonifen	Oral 1 d Acute	LD_{50} **> 2000** mg/kg bw/d	EFSA, 2008
		Dietary 8 d Short-term	5-day LD_{50} > 1027 mg/kg bw/d	
Japanese quail	Aclonifen	Oral 1 d Acute	LD_{50} > 15000 mg/kg bw/d	
		Dietary Reproductive toxicity	NOEL **> 141** mg/kg bw/d	
Atlantic canary	Aclonifen	Oral 1 d Acute	LD_{50} > 15000 mg/kg bw/d	
Bobwhite quail	Flufenacet	Oral 1 d Acute	LD_{50} = 1608 mg/kg bw/d	SANCO/7469/VI/98-Final, 03 July 2003
Mallard duck	Flufenacet	Subchronic and reproductive toxicity	NOEL = 88 ppm*	SANCO/7469/VI/98-Final, 03 July 2003

***** Although in DAR and in the Review Report, the NOEL was expressed in ppm, the RMS of the active substance (France) agreed the converted daily dose value of 9.87 mg/kg bw in a zonal assessment.

9.2.1.1 Justification for new endpoints

EU agreed endpoints were used in the risk assessment. No deviations were made.

9.2.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Birds and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438. To achieve a concise risk assessment, the risk envelope approach is applied.

9.2.2.1 First-tier assessment (screening/generic focal species)

Exposure to standard generic indicator species was estimated according to the 'Guidance of EFSA – Risk assessment for Birds and Mammals' (EFSA 2009). The proposed use of GLOB1310aH as a herbicide in cereals is pre-emergence and early post-emergence. The appropriate exposure scenario for a pre-emergence application in cereals is deemed to be bare soil.

The results of the acute and reproductive first-tier risk assessments for the screening step are summarised in the following tables. The use of 2L/ha at pre-emergence (uses 7-12) covers the use of 1.5L/ha of

GLOB1310aH at pre-emergence.

Table 0-2: Aclonifen: screen assessment and first tier of the acute and long term/reproductive risk for birds due to the use of 2L/ha GLOB1310aH in cereals at pre-emergence

Intended use	Bare soil (to represent the pre-emergence use in cereals) 2L formulation/ha				
Active substance/product	Aclonifen				
Application rate (g/ha)	1 × 1.08 kg/ha				
Acute toxicity (mg/kg bw)	2000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening step					
Bare soil	Small granivorous bird	25.3 ~~24.7~~	1	27.32 ~~26.68~~	73.19 ~~75.0~~
First Tier					
Bare soil, BBCH<10	Small granivorous bird	~~24.7~~ 25.3	1	~~26.68~~ 27.32	~~74.97~~ 73.19
Bare soil, BBCH<10	Small omnivorous bird “lark”	17.4	1	18.79	106.43
Bare soil, BBCH<10	Small insectivorous bird “wagtail”	10.9	1	11.77	169.89
Acute toxicity (mg/kg bw)	> 1027				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening step					
Bare soil	Small granivorous bird	25.3	1	27.32	37.6
Reprod. toxicity (mg/kg bw/d)	141				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening step					
Bare soil	Small granivorous bird	11.4	1 x 0.53	6.53	21.61
First Tier					
Bare soil, BBCH<10	Small granivorous bird	11.4	1 x 0.53	6.53	21.61
Bare soil, BBCH<10	Small omnivorous bird “lark”	8.2	1 x 0.53	4.69	30.04
Bare soil, BBCH<10	Small insectivorous bird “wagtail”	5.9	1 x 0.53	3.37	41.75

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The TER_a and TER_{lt} values for Aclonifen are greater than the Annex VI triggers of respectively 10 and 5, indicating that GLOB1310aH presents low acute risk and no unacceptable long-term risk to birds follow-

ing pre-emergence application of GLOB1310aH at 2L/ha and at 1.5L/ha in winter cereals. Therefore, further risk assessments are not required.

Table 0-3: Flufenacet: screen assessment and first tier of the acute and long-term/reproductive risk for birds due to the use of 2 L/ha GLOB1310aH in cereals at pre-emergence

Intended use	Bare soil (to represent the pre-emergence use in cereals)				
Active substance/product	Flufenacet				
Application rate (g/ha)	1 × 120 g/ha				
Acute toxicity (mg/kg bw)	1608				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening step					
Bare soil	Small granivorous bird	25.3 ~~24.7~~	1	3.03 ~~2.964~~	529.64 ~~542.51~~
First Tier					
Bare soil, BBCH<10	Small granivorous bird	~~24.7~~ 25.3	1	~~2.964~~ 3.03	~~542.51~~ 529.64
Bare soil, BBCH<10	Small omnivorous bird “lark”	17.4	1	2.09	770.11
Bare soil, BBCH<10	Small insectivorous bird “wagtail”	10.9	1	1.31	1229.36
Reprod. toxicity (mg/kg bw/d)	9.87				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening step					
Bare soil	Small granivorous bird	11.4	1 x 0.53	0.725	13.61
First Tier					
Bare soil, BBCH<10	Small granivorous bird	11.4	1 x 0.53	0.725	13.61
Bare soil, BBCH<10	Small omnivorous bird “lark”	8.2	1 x 0.53	0.521	18.93
Bare soil, BBCH<10	Small insectivorous bird “wagtail”	5.9	1 x 0.53	0.375	26.30

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The TER_a and TER_{lt} values for Flufenacet are greater than the Annex VI triggers of respectively 10 and 5, indicating that GLOB1310aH presents low acute risk and no unacceptable long-term risk to birds following pre-emergence application of GLOB1310aH at 2L/ha and at 1.5L/ha in winter cereals. Therefore, further risk assessments are not required.

9.2.2.2 Combined risk assessment

Since GLOB1310aH contains 2 active ingredients a combined risk assessment was performed.

According to Appendix B of the Guidance Document on the Risk Assessment for birds and mammals, the basic concept of the risk assessment is that animals are exposed to residues of the active substances in the environment. Thus the assessment of GLOB1310aH is not an assessment of the formulation as such, but an assessment of the effects of an exposure to a mixture of active substances in the environment, resulting from the use of the formulation. Toxicity studies for birds with formulated products are typically not available.

For the assessment of acute effects, a surrogate LD_{50} is calculated. A model often used to estimate the toxicity mixtures is the assumption of dose/concentration additivity of toxicity (Finney approach of concentration additivity of toxicity (Finney, D.J., 1948 and 1971).

The following formula is used to derive a surrogate LD_{50} for the mixture of active substances with known toxicity assuming additivity:

$$LD_{50}(\text{mix}) = \left(\sum_i \frac{X(\text{a.s.}_i)}{LD_{50}(\text{a.s.}_i)} \right)^{-1}$$

With:

$X(\text{a.s.}_i)$ = fraction of active substance [i] in the mixture;
(please note that the sum $\sum X(\text{a.s.}_i)$ must be 1)

$LD_{50}(\text{a.s.}_i)$ = acute toxicity value for active substance [i]

Then, the LD_{50} mix for birds of GLOB1310aH amounts to 1952.4 mg/kg bw/d (=1/[(0.9/2000)+(0.1/1608)]).

The avian "tox per fraction" for the acute exposure was calculated as well. The table below summaries the results.

	Aclonifen	Flufenacet
Content in the formulation GLOB1310aH	540 g/L	60g/L
Fraction in mixture	0.9	0.1
LD_{50} (mg/kg bw)	2000	1608
Tox per fraction	2000/0.9= 2222.2	1608/0.1= 16080
Predicted LD_{50} mixture	1952.4	
~~Deviation (%)~~	~~13.82~~	~~8.52~~

The deviation of the a.s.tox per fractionin % from the mix tox per fraction.

Substance	Toxicity	Fraction	Tox per fraction	Deviation
Birds				
Mix	$LD_{50\ mix}$ = 1952.4	1	1952.4	
Aclonifen	LD_{50} = 2000	0.9	2222	+14%
Flufenacet	LD_{50} = 1608	0.1	16080	+724%

~~The "tox per fraction" of active substances and of mix LD50 did not deviate by less than 10% for Aclonifen. For flufenacet a deviation lower than 10% (8.52%) was observed. Thus, according to the EFSA guidance (2009) flufenacet drives the risk assessment for birds and the risk assessment for this active substance (flufenacet) covers the combined risk assessment for GLOB1310aH and no further considerations are necessary. However, for completeness sake~~ aAn acute first tier assessment for birds is done below with the predicted LD_{50} (mix).

Table 0-4: GLOB1310aH: Screen assessment and first tier of the acute and long-term/reproductive risk for birds due to the use of 2 L/ha GLOB1310aH in cereals at pre-emergence (the use of 2L/ha covers the use of 1.5L/ha)

Intended use	Bare soil (to represent the pre-emergence use in cereals)				
Active substance/product	GLOB1310aH				
Application rate (g/ha)	1 × 1200 g a.s./ha (2L GLOB1310aH/ha: 1080 g Aclonifen/ha + 120 g Flufenacet/ha)				
Acute toxicity (mg/kg bw)	1952.4				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening step					
Bare soil	Small granivorous bird	25.3 ~~24.7~~	1	30.36 ~~29.64~~	64.31 ~~65.87~~
First Tier					
Bare soil, BBCH<10	Small granivorous bird	~~24.7~~ 25.3	1	~~29.64~~ 30.36	~~65.87~~ 64.31
Bare soil, BBCH<10	Small omnivorous bird “lark”	17.4	1	20.88	93.51
Bare soil, BBCH<10	Small insectivorous bird “wagtail”	10.9	1	13.08	149.27

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Using the same approach, also a NOEL (mix) was calculated which amounts to 60.55 mg/kg bw/d (=1/[(0.9/141)+(0.1/9.87)]).

The avian “tox per fraction” for the long term exposure was calculated as well. The table below summaries the results.

	Aclonifen	Flufenacet
Content in the formulation GLOB1310aH	540 g/L	60g/L
Fraction in mixture	0.9	0.1
NOEL (mg/kg bw/d)	141	9.87
Tox per fraction	141/0.9= 156.7	9.87/0.1= 98.7
Predicted LD_{50} mixture	60.55	
Deviation (%)	158.7	63.0

The “tox per fraction” of active substances and of mixNOEL did not deviate by less than 10% for Aclonifen or flufenacet. Thus, according to the EFSA guidance (2009) there is no clear toxicity driver for birds. Thus a long term first tier assessment for birds is done below with the predicted NOEL (mix). The calculations are done for the use of 2L/ha, which covers the use of 1.5L/ha.

Table 0-5: GLOB1310aH: Screen assessment and first tier of the acute and long-term/reproductive risk for birds due to the use of 2 L/ha GLOB1310aH in cereals at pre-emergence

Intended use	Bare soil (to represent the pre-emergence use in cereals)
Active substance/product	GLOB1310aH
Application rate (g/ha)	1 × 1200 g a.s./ha (2L GLOB1310aH/ha: 1080 g Aclonifen/ha + 120 g

	Flufenacet/ha)				
Reprod. toxicity (mg/kg bw/d)	60.55				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening step					
Bare soil	Small granivorous bird	11.4	1 x 0.53	7.25	8.35
First Tier					
Bare soil, BBCH<10	Small granivorous bird	11.4	1 x 0.53	7.25	8.35
Bare soil, BBCH<10	Small omnivorous bird "lark"	8.2	1 x 0.53	5.21	11.61
Bare soil, BBCH<10	Small insectivorous bird "wagtail"	5.9	1 x 0.53	3.75	16.13

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

In addition, a combined risk assessment for sublethal effects was performed as well using the following equation and assuming a direct proportionality of the TER to the NOEL:

$$TER(mix) = \left(\sum_i \frac{1}{TER(a.s._i)} \right)^{-1}$$

where:

$TER_{(a.s.i)}$= calculated TER for the active substance i

Then, the TER long-term mix of GLOB1310aH – pre-emergence (2L/ha) for birds at screening step: 1/[(1/21.61)+(1/13.61)]= 8.35

9.2.2.3 Higher-tier risk assessment

Not required as the risk was acceptable at a lower tier.

9.2.2.4 Drinking water exposure

When necessary, the assessment of the risk for birds due to uptake of contaminated drinking water is conducted for a small granivorous bird with a body weight of 15.3 g (*Carduelis cannabina*) and a drinking water uptake rate of 0.46 L/kg bw/d (*cf.* Appendix K of EFSA/2009/1438).

Leaf scenario

Since GLOB1310aHis not intended to be applied on leafy vegetables forming heads or crop plants with comparable water collecting structures at principal growth stage 4 or later, the leaf scenario does not have to be considered.

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

Aclonifen

As the K(f)oc exceeds 500 L/kg, Aclonifen belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use of 2L GLOB1310aH also covers the risk for birds from all other intended uses.

Effective application rate Aclonifen (g/ha)* =	1080				Trigger
Acute toxicity (mg/kg bw) =	~~1027~~ 2000	quotient	=	1.~~08~~5	3000
Reprod. toxicity (mg/kg bw/d) =	141	quotient	=	7.66	3000

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) doesn't exceed the critical value of 3000, no quantitative risk assessment (calculation of TER values) is necessary.

Flufenacet

With a K(f)oc of 187 mL/g, Flufenacet belongs to the group of less sorptive substances. The assessment fot the use of GLOB1310aH at 2L/ha (120 g flufenacet/ha) also covers the risk for birds from the other intended uses:

Effective application rate Flufenacet (g/ha) =	120				Trigger
Acute toxicity (mg/kg bw) =	1608	quotient	=	0.075	50
Reprod. toxicity (mg/kg bw/d) =	9.87	quotient	=	12.16	50

No specific calculations of exposure and TER are necessary as the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of the less sorptive substances-flufenacet.

9.2.2.5 Effects of secondary poisoning

The log P_{ow} of Aclonifen amounts to 4.37 and thus exceeds the trigger value of 3. It was therefore necessary to consider the risk from secondary poisoning further. The risk assessment is provided below.

The log Pow of flufenacet (log Pow = 3.20) exceed the trigger value of 3. A risk assessment for effects due to secondary poisoning is then required for this substance.

Risk assessment for earthworm-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous birds is assessed for a bird of 100 g body weight with a daily food consumption of 104.6 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

As indicated in the Working document on Risk Assessment of Plant Protection Products in the Central Zone – Ecotoxicology (version 1.0, May 2021), for the risk assessment, secondary poisoning of birds through earthworms, the dry soil approach is followed. The residue in earthworms (i.e. PEC earthworm) is estimated by multiplying the appropriate PEC soil (see below) with the BCF earthworm:

− For Non-persistent substances: take PECsoil,twa,21 days (or PECsoil,max as a worst-case), this is the case of flufenacet
− For Persistent substances: take PECsoil,twa,21 days + PECsoil,plateau (or PECsoil,max as a worst-

case), this is the case of Aclonifen

Aclonifen

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 2 also covers the risk for birds from all other intended uses in group 1 (see 0).

Table 0-6: Assessment of the risk for earthworm-eating birds due to exposure to Aclonifen via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals (2L/ha)

Parameter	Aclonifen	comments
PEC_{soil} (max) (mg/kg soil)	1.981	dRR Part B8 Annex point 8.7.2
log P_{ow} / P_{ow}	4.37 / 23442	EFSA Scientific Report, 2008
Koc	5318	Worst-case value (n = 3)
foc	0.02	Default
BCF_{worm}	2.65	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	~~4.17~~ 5.26	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	~~4.39~~ 5.52	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	141	EFSA, 2008
TER_{lt}	25.6~~55~~	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

The TER_{lt} values are greater than the Annex VI trigger of 5 for the earthworm-eating birds, indicating that Aclonifen poses low long-term risk to these birds following application of GLOB1310aHat the proposed rate in cereals.

Flufenacet

Table 0-7: Assessment of the risk for earthworm-eating birds due to exposure to Flufenacet via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals (use 3, 2L GLOB1310aH/ha, covers all requested uses)

Parameter	Flufenacet	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.1402	dRR Part B8 Annex point 8.7.2
log P_{ow} / P_{ow}	3.2/1585	Review Report 2003
Koc	187	Geomean (n = 3)
foc	0.02	Default
BCF_{worm}	5.31	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.74	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.781	$DDD = PEC_{worm} \times 1.05$
NOEL (mg/kg bw/d)	9.87	EFSA, 2008
TER_{lt}	12.62	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

Combined risk assessment

Since GLOB1310aH contains 2 active ingredients, a risk assessment for the mixture of active substances was performed using the NOEL (mix) of 60.55 mg/kg bw/d. The calculations are done only for the use of 2.0L/ha which covers the use of 1.5L/ha.

$$TER(mix) = \left(\sum_i \frac{1}{TER(a.s._i)} \right)^{-1}$$

where:

$TER_{(a.s.i)}$= calculated TER for the active substance i

$TER_{mix} = (1/25.55 + 1/12.62)^{-1} = (0.0141 + 0.158)^{-1} = 8.44$

Risk assessment for fish-eating birds via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous birds is assessed for a bird of 1000 g body weight with a daily food consumption of 159 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

As indicated in the Working document on Risk Assessment of Plant Protection Products in the Central Zone – Ecotoxicology (version 1.0, May 2021), the Central Zone decided that in a screening step (i.e. before first tier) the lowest acceptable surface water concentration for aquatic organisms should be used.

Aclonifen

The lowest RAC for aquatic organisms to aclonifen is 5 µg/L, for long-term toxicity fish.

Table 0-8: Screening step of the risk for fish-eating birds due to exposure to Aclonifen via bioaccumulation in fish (secondary poisoning) for the intended use in cereals (2 L GLOB1310aH/ha)

Parameter	Aclonifen	comments
RAC aclonifen aquatic organisms (mg/L)	0.0005	RAC aclonifen aquatic organisms (mg/L), long-term toxicity fish
BCF_{fish}	2896	EFSA, 2008
PEC_{fish}	1.448	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.2302	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	141	EFSA, 2008
TER_{lt}	612.42	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

Additionally, the risk for fish-eating birds due to exposure to Aclonifen was also calculated considering the calculated the max PECsw and the 21d PECsw at Step 3.

Table 0-9: Assessment of the risk for fish-eating birds due to exposure to Aclonifen via bioaccumulation in fish (secondary poisoning) for the intended use in cereals (2 L GLOB1310aH/ha)

Parameter	Aclonifen	comments
PEC_{sw} (21d) (mg/L)	~~0.003519~~ 0.003486	~~dRR Part B8 Annex point 8.9.2~~ ~~Max 21d PECsw Step 3 (3.519 µg/L, D1 dieth)~~ Overall worst case FOCUS Step 3 TWA 21-d PECsw for an application rate of 1080 g a.s./ha
BCF_{fish}	2896	EFSA, 2008
PEC_{fish}	10.10~~9~~	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	1.6059~~203~~	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	141	EFSA, 2008
TER_{lt}	87.02	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

Flufenacet

The lowest RAC for aquatic organisms to flufenacet is 0.243 µg/L, for long-term toxicity to aquatic macrophytes *Lemna*.

Table 0-10: Assessment of the risk for fish-eating birds due to exposure to Flufenacet via bioaccumulation in fish (secondary poisoning) for the intended use in cereals (2L/ha)

Parameter	Flufenacet	comments
RAC flufenacet aquatic organisms (mg/L)	0.000243	RAC flufenacet aquatic organisms (mg/L), aquatic macrophytes
BCF_{fish}	71.4	Review report, 2003
PEC_{fish}	0.017	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.003	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	9.87	EFSA, 2008
TER_{lt}	3577	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

Additionally, the risk for fish-eating birds due to exposure to Flufenacet was also calculated considering the calculated the max PECsw and the 21d PECsw at Step 3.

Table 0-11: Assessment of the risk for fish-eating birds due to exposure to Flufenacet via bioaccumulation in fish (secondary poisoning) for the intended use in cereals (2L/ha)

Parameter	Flufenacet	comments
PEC_{sw} (21d) (mg/L)	~~0.003613~~ 0.00348	~~dRR Part B8 Annex point 8.9.2~~ ~~Max 21d PECsw Step 3 (3.613 µg/L, D1 dieth)~~ Overall worst case FOCUS Step 3 TWA 21-d PECsw for an application rate of 120 g a.s./ha
BCF_{fish}	71.4	Review report, 2003

Parameter	Flufenacet	comments
PEC_{fish}	0.24857	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.03941	$DDD = PEC_{fish} \times 0.159$
NOEL (mg/kg bw/d)	9.87	EFSA, 2008
TER_{lt}	2490.6	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

Combined risk assessment

$$TER(mix) = \left(\sum_i \frac{1}{TER(a.s._i)} \right)^{-1}$$

where:

$TER_{(a.s.i)}$= calculated TER for the active substance i

$TER_{mix} = (1/87.02 + 1/2490.6)^{-1} = 64.563.9$

9.2.2.6 Biomagnification in terrestrial food chains

Not relevant.
The results of the ADME studies indicate that Aclonifen has a low bioaccumulation potential. It is therefore assumed that there will be no biomagnification along the food chain.

Low potential for accumulation in animal tissue was concluded in the EU review of flufenacet. Since the bioaccumulation potential of flufenacet is low no further assessment on biomagnification is required.

9.2.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant. GLOB1310aHis intended for use as a foliar spray.

9.2.4 Overall conclusions

The TER_a and TER_{lt} values exceed the triggers of 10 and 5 for the acute and long-term assessment respectively, indicating that Aclonifen and Flufenacet do not pose an acute nor a long-term risk to wild birds after the use of GLOB1310aH according to the intended GAP.

The long-term TERs for secondary poisoning via earthworms and fish based on 21 days TWA PEC_{soil} and PEC_{sw} values were all above the Annex VI trigger of 5, indicating acceptable risks.

Review comments:

The risk assessment to birds was performed in accordance with the recommendation of Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA (EFSA Journal 2009; 7(12):1438).

The results of the ‘screening phase’ acute dietary risk assessment and Tier-1 long term dietary risk assessment - Toxicity Exposure Ratios (TER_A and TER_{LT}) were calculated taking into account the EU agreed endpoints for most sensitive species for the active substance and using the EFSA Bird and

Mammal risk assessment calculator for the higher predicted application rate than it is foreseen in GAP exceeding the trigger set by Commission regulation (EU) 546/2011 for acceptability of effects. Revealed that there is no potential of risk for birds resulting from acute and long-term exposure to active substance following use of GLOB1310aH in compliance with proposed GAP.

A quantitative drinking water risk assessment is not triggered for the proposed use pattern of GLOB1310aH according to EFSA/2009/1438 criteria and therefore the risk to birds via drinking water is acceptable.

Since the acceptability criterion of TER ≥ 5 is achieved for all active substances, an acceptable risk to earthworm-eating and fish-eating birds via secondary poisoning can be concluded for all intended uses.

No risk mitigation measures are required.

Conclusion

According to the performed risk assessment there is no potential of risk to birds resulting from exposure to active substance following use of Glosset Ace (GLOB1310aH) in compliance with proposed GAP.

9.3 Effects on terrestrial vertebrates other than birds (KCP 10.1.2)

The risk assessment to mammals was performed in accordance with the recommendations of the Guidance Document on Risk Assessment for Birds and Mammals (EFSA, 2009).

9.3.1 Toxicity data

Mammalian toxicity studies have been carried out with Aclonifen and Flufenacet. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on mammals of GLOB1310aHwere not evaluated as part of the EU assessment of Aclonifen or Flufenacet. However, further data on GLOB1310aHare not relevant as active substances data on toxicity to terrestrial vertebrates other than birds are used and additional formulation data are not considered essential.

Mammals are typically exposed to dry residues on their food items following the dilution and spraying of the formulated product. During these processes, much of the formulation constituents are likely to be lost by volatilisation. Therefore, where oral exposure is the main route of exposure, toxicity data for the active substance are used in preference to data from tests with the formulated material. Exposure to GLOB1310aH via dermal and inhalation routes is considered unlikely, since at the time of application and for a short period thereafter, most wild mammals will leave the immediate vicinity of spray operations in response to the human disturbance. Therefore, all relevant data were assessed in the EU review. Risk assessments for GLOB1310aH with the proposed use pattern are provided here and are considered adequate.

Table 0-1: Endpoints and effect values relevant for the risk assessment for mammals

Species	Substance	Exposure System	Results	Reference
Rat and mouse	Aclonifen	Oral 1 d Acute	LD_{50} > 5000 mg/kg bw/d	EFSA, 2008
Rat	Aclonifen	Dietary Reproductive toxicity Two-generation study	NOAEL = 8 mg/kg bw/d 35 mg/kg bw/d*	
Rat	Flufenacet	Oral Acute	LD50 female = 589 mg/kg bw	EC review report 7469/VI/98-Final (2003)
Rat	Flufenacet	Dietary Reproductive toxicity Multi-generation study	NOAEL = 25 mg/kg bw/d	

*Please se justification in section 9.3.1.1

9.3.1.1 Justification for new endpoints

9.3.2 Risk assessment for spray applications

The risk assessment is based on the methods presented in the Guidance Document on Risk Assessment for Mammals and Mammals on request from EFSA (EFSA Journal 2009; 7(12): 1438; hereafter referred to as EFSA/2009/1438).

9.3.2.1 First-tier assessment (screening/generic focal species)

The results of the acute and reproductive screening and first-tier risk assessments are summarised in tables below.

Table 0-2: Aclonifen: screening assessment and first tier of the acute and long-term/reproductive risk for mammals due to the use of Aclonifen in cereals at pre-emergence 2L/ha

Intended use	Bare soil (to represent the pre-emergence use in cereals)				
Active substance/product	Aclonifen				
Application rate (g/ha)	1 × 1.08 kg/ha				
Acute toxicity (mg/kg bw)	5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening					
Bare, soil	Small granivorous mammal	14.4	1	15.55	321.50
First Tier					
Bare soil, BBCH<10	Small omnivorous mammal “mouse”	14.3	1	15.44	323.75

Reprod. toxicity (mg/kg bw/d)	8				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening					
Bare soil	Small granivorous mammal	6.6	1 x 0.53	3.77	**2.12**
First Tier					
Bare soil, BBCH<10	Small omnivorous mammal "mouse"	5.7	1 x 0.53	3.26	**2.45**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 0-3: Aclonifen: screening assessment and first tier of the acute and long-term/reproductive risk for mammals due to the use of Aclonifen in cereals at pre-emergence 1.5L/ha

Intended use	Bare soil (to represent the pre-emergence use in cereals)				
Active substance/product	Aclonifen				
Application rate (g/ha)	1 × 0.810 kg/ha				
Acute toxicity (mg/kg bw)	5000				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening					
Bare, soil	Small granivorous mammal	14.4	1	11.66	428.67
First Tier					
Bare soil, BBCH<10	Small omnivorous mammal "mouse"	14.3	1	11.58	431.67
Reprod. toxicity (mg/kg bw/d)	8				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening					
Bare soil	Small granivorous mammal	6.6	1 x 0.53	2.83	**2.82**
First Tier					
Bare soil, BBCH<10	Small omnivorous mammal "mouse"	5.7	1 x 0.53	2.45	**3.26**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The TER_a value is higher than the Annex VI trigger value of 10, indicating that GLOB1310aH poses low acute risk to mammals following application of 2.0L/ha or 1.5L/ha of GLOB1310aH at pre-emergence to winter cereals. Therefore no further considerations are necessary.

The TER_{lt} value is not higher than the Annex VI trigger value of 5, indicating that GLOB1310aH poses undue chronic risk to mammals following application of 2.0L/ha or 1.5L/ha of GLOB1310aH at pre-

emergence to winter cereals. Therefore further considerations are necessary.

Flufenacet

Table 0-4: Flufenacet: screening assessment and first tier of the acute and long-term/reproductive risk for mammals due to the use of GLOB1310aH in cereals at pre-emergence 2L/ha

Intended use	Bare soil (to represent the pre-emergence use in cereals)				
Active substance/product	Flufenaet				
Application rate (g/ha)	1 × 120 g/ha				
Acute toxicity (mg/kg bw)	589				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening					
Bare, soil	Small granivorous mammal	14.4	1	1.728	340.86
First Tier					
Bare soil, BBCH<10	Small omnivorous mammal "mouse"	14.3	1	1.72	343.24
Reprod. toxicity (mg/kg bw/d)	25				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening					
Bare soil	Small granivorous mammal	6.6	1 x 0.53	0.42	59.56
First Tier					
Bare soil, BBCH<10	Small omnivorous mammal "mouse"	5.7	1 x 0.53	0.36	68.96

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

Table 0-5: Flufenacet: screening assessment and first tier of the acute and long-term/reproductive risk for mammals due to the use of GLOB1310aH in cereals at pre-emergence 1.5L/ha

Intended use	Bare soil (to represent the pre-emergence use in cereals)				
Active substance/product	Flufenaet				
Application rate (g/ha)	1 × 90 g/ha				
Acute toxicity (mg/kg bw)	589				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening					
Bare, soil	Small granivorous mammal	14.4	1	1.30	454.48
First Tier					
Bare soil, BBCH<10	Small omnivorous mammal	14.3	1	1.29	457.65

	"mouse"				
Reprod. toxicity (mg/kg bw/d)	25				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening					
Bare soil	Small granivorous mammal	6.6	1 x 0.53	0.314	79.41
First Tier					
Bare soil, BBCH<10	Small omnivorous mammal "mouse"	5.7	1 x 0.53	0.271	91.95

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The TER_a and TER_{lt} value is higher than the Annex VI trigger value of 10 and 5, indicating that GLOB1310aH poses low acute and chronic risk to mammals following application of 2.0L/ha or 1.5L/ha of GLOB1310aH at pre-emergence to winter cereals. Therefore no further considerations are necessary.

9.3.2.2 Higher-tier risk assessment

Aclonifen

Higher tier is necessary for the use of GLOB1310aH due to an unacceptable chronic risk to small omnivorous mammal "mouse".

Small mammals like the woodmouse prefer low vegetation as feeding habitat which provides cover for predators, they will only spend very little time on bare soil (which is the crop situation for the risk assessment at early post-emergence). In Addendum 4 to the DAR of 13 December 2011, confirmatory data were submitted to investigate the real portion of time spend in bare soil fields by small mammals. Three studies (Barfknecht 2006-2010, Funkenhausen & Giessing 2009) were submitted, in which different freshly drilled maize, sugar beet and oilseed rape fields in Central and Southern Europe were monitored for the occurrence of wild small mammals as the wood mouse:

- AIII 10.3.2. Barfknecht, 2010. Generic field monitoring of birds and mammals on maize and beet fields in Austria – an excerpt from the GLP study WFC/FS017, performed by Christian Wolf 2005.
- AIII 10.3.2. Barfknecht, 2006. Generic field monitoring of mammals in freshly drilled oilseed rape fields in summer in Germany.
- AIII 10.3.2. Funkenhausen A. and Giessing B., 2009. Exposure of mammals in maize fields in France – Attractiveness of maize fields and relevant species.

These studies started some weeks before drilling and were completed when BBCH stage 14 till 16 was reached. Monitoring was done by live trapping and continuously radio tracking of different species during which the location, habitat and behaviour was recorded.

The live trapping results revealed that the uncultivated plain fields with last years crop residues were more attractive to small mammals than the same fields once cultivated and drilled, as these uncultivated plain fields provide more protection against predators. Wood mouse seemed to be a focal species within freshly drilled maize, sugar beet and oilseed rape fields, although the surrounding habitats proved to be more attractive. The highest proportion of time was spent in the habitat hedgerow/shrub and the majority of individuals used a mixed habitat, characterised by structures like grassland, bushes and trees, during potential foraging time.

Freshly sown fields of winter cereals were not tested in these studies but the tested maize fields (Wolf, 2005, reevaluated for mammals by Brafknecht (2010)) can be considered as a surrogate as all these fields represent a bare drilled soil situation at the time of application of Aclonifen and therefore are not attractive to small mammals as they will provide not any cover against predators. The data presented on sugar beet fields might not be appropriate since woodmice are known digging out beet seed pills. Therefore the focus should be on maize.

In this study of Wolf (2005) reevaluated for mammals by Brafknecht (2010) the mean proportion of active time within the field were 0% and 2% for maize. While, the mean within the sugarbeet (freshly drilled and germinated) was 38% and 32%. Therefore, a refined PT of 2% is adequate to use as refinement. However, to provide a conservative value (since the study is on maize and during spring), a PT of 38% obtained in sugarbeet is used as a surrogate for winter cereals knowing that this is significantly overestimating the real portion of time spend by woodmice in drilled fields. Moreover, this PT of 0.38 was accepted by the RMS Germany (DE, Addendum 4 to DAR, 2011). On top of this, there is an ongoing field monitoring study on woodmice in winter cereals field during the autumn-winter period (sponsored by the applicant). The preliminary results shows a PT lower than 0.38. The results will be submitted as soon as the study.

The refined calculations for woodmice are shown below.

Table 0-6: Aclonifen: Higher tier of long-term/reproductive risk for mammals due to the use of GLOB1310aH in cereals at pre-emergence (2.0 and 1.5L/ha)

Intended use	Bare soil (pre-emergence application in cereals)					
Active substance/product	Aclonifen					
Application rate (g/ha)	1 × 1.080 kg/ha					
Reprod. toxicity (mg/kg bw/d)	8					
TER criterion	5					
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening						
Bare soil	Small granivorous mammal	6.6	1 x 0.53	0.38	1.435	5.57
First Tier						
Bare soil, BBCH<10	Small omnivorous mammal "mouse"	5.7	1 x 0.53	0.38	1.239	6.45
Intended use	Bare soil (pre-emergence application in cereals)					
Active substance/product	Aclonifen					
Application rate	1 × 0.810 kg/ha					
Reprod. toxicity (mg/kg bw/d)	8					
TER criterion	5					
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening						
Bare soil	Small granivorous mammal	6.6	1 x 0.53	0.38	1.076	7.43
First Tier						
Bare soil, BBCH<10	Small omnivorous mammal "mouse"	5.7	1 x 0.53	0.38	0.929	8.60

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The TER_{lt} value is higher than the Annex VI trigger value of 5, indicating that GLOB1310aH poses low chronic risk to mammals following application of 1.5L/ha and 2L/ha of GLOB1310aH at pre-emergence to winter cereals.

9.3.2.3 Combined risk assessment

Since GLOB1310aH contains 2 active ingredients a combined risk assessment was performed.

Following Appendix B (step 1) in EFSA/2009/1438 a surrogate LD_{50} is calculated based on the assumption of dose additivity. A combined acute risk assessment is not required if for one active substance the deviation between 'tox per fraction (a.s.)' and 'tox per fraction (mix)' is ≤ 10 % as in that case the risk is covered by the assessment for that active substance.

The following formula is used to derive a surrogate LD_{50} for the mixture of active substances with known toxicity assuming additivity:

$$LD_{50}(\text{mix}) = \left(\sum_i \frac{X(\text{a.s.}_i)}{LD_{50}(\text{a.s.}_i)} \right)^{-1}$$

With:

$X(\text{a.s.}_i)$ = fraction of active substance [i] in the mixture; (please note that the sum $\sum X(\text{a.s.}_i)$ must be 1)

$LD_{50}(\text{a.s.}_i)$ = acute toxicity value for active substance [i]

Then, the LD_{50} mix of GLOB1310aH for mammals amounts to 2858.95 mg/kg bw/d (=1/[(0.9/5000)+(0.01/589)]).

The "tox per fraction" for the acute exposure of mammals was calculated as well. The table below summaries the results.

	Aclonifen	Flufenacet
Content in the formulation GLOB1310aH	540 g/L	60g/L
Fraction in mixture	0.9	0.1
LD_{50} (mg/kg bw)	5000	589
Tox per fraction	5000/0.9= 5555.5	589/0.1= 5890
Predicted LD_{50} mixture	2858.95	
Deviation (%)	94.3	106

The "tox per fraction" of active substances and of mix LD50 did not deviate by less than 10% for Aclonifen and flufenacet. Thus, none of the active substances clearly drives the risk assessment and a combined acute first tier assessment for mammals is done below with the predicted LD50 (mix).

Table 0-7: GLOB1310aH: screening assessment and first tier of the acute risk for mammals due to the use of GLOB1310aH in cereals at pre-emergence 2L/ha and 1.5L/ha

Intended use	Bare soil (to represent the pre-emergence use in cereals)
Active substance/product	GLOB1310aH
Application rate (g/ha)	1 × 1200 g a.s./ha (2L GLOB1310aH/ha: 1080 g Aclonifen/ha +120 g Flufenacet/ha)
Acute toxicity (mg/kg bw)	2858.95

TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening					
Bare, soil	Small granivorous mammal	14.4	1	17.28	167.76
First Tier					
Bare soil, BBCH<10	Small omnivorous mammal "mouse"	14.3	1	17.16	168.94
Intended use	Bare soil (to represent the pre-emergence use in cereals)				
Active substance/product	GLOB1310aH				
Application rate (g/ha)	1 × 900 g a.s./ha (1.5L GLOB1310aH/ha: 810 g Aclonifen/ha +90 g Flufenacet/ha)				
Acute toxicity (mg/kg bw)	2858.95				
TER criterion	10				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_{90}**	**MAF_{90}**	**DDD_{90} (mg/kg bw/d)**	**TER_a**
Screening					
Bare, soil	Small granivorous mammal	14.4	1	12.96	223.68
First Tier					
Bare soil, BBCH<10	Small omnivorous mammal "mouse"	14.3	1	12.87	225.25

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

The first TER_A value is above the trigger of 5 for short-term exposure, indicating an acceptable acute risk for mammals from the use at pre-emergence of 1.5L/ha and 2.0L/ha of GLOB1310aH in winter cereals.

In addition, a combined risk assessment for acute effects was performed as well using the following equation and assuming a direct proportionality of the TER to the EC:

$$TER(mix) = \left(\sum_i \frac{1}{TER(a.s._i)} \right)^{-1}$$

where:

$TER_{(a.s.i)}$= calculated TER for the active substance i

The TER acue mix of GLOB1310aH – pre-emergence (2L/ha) for mammals at first step:
1/[(1/323.75)+(1/343.24)]= 166

The TER acute mix of GLOB1310aH – pre-emergence (1.5L/ha) for mammals at first step:
1/[(1/431.67)+(1/457.65)]= 222.14

Using the same approach, also a NOEL (mix) was calculated which amounts to 8.58 mg/kg bw/d (=1/[(0.9/8)+(0.1/25)]).

The "tox per fraction" for the long term exposure of mammals was calculated as well. The table below summaries the results.

	Aclonifen	Flufenacet
Content in the formulation GLOB1310aH	540 g/L	60g/L

Fraction in mixture	0.9	0.1
NOEL (mg/kg bw)	8	25
Tox per fraction	8/0.9= ~~5555.58~~.9	25/0.1= ~~5890~~250
Predicted NOEL mixture	8.58	
Deviation (%)	~~3.55~~ 4	~~281.25~~ 2813

The tox per fraction for Aclonifen deviates by ~~3.55~~ 4% from the mixture toxicity, indicating that Aclonifen contributes to more than 90% to the toxicity of the formulation. Consequently, the reproductive risk assessments can be based on the Aclonifen only, covering flufenacet which only contribute marginally to mixture toxicity. However, for completeness sake the calculations are performed and presented below.

Table 0-8: GLOB1310aH: screening assessment and first tier of the long-term/reproductive risk for mammals due to the use of GLOB1310aH in cereals at pre-emergence 2L/ha and 1.5L/ha

Intended use	Bare soil (to represent the pre-emergence use in cereals)				
Active substance/product	GLOB1310aH				
Application rate (g/ha)	1 × 1200 g a.s./ha (2L GLOB1310aH/ha: 1080 g Aclonifen/ha +120 g Flufenacet/ha)				
Reprod. toxicity (mg/kg bw/d)	8.58				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening					
Bare soil	Small granivorous mammal	6.6	1 x 0.53	4.197	**2.04**
First Tier					
Bare soil, BBCH<10	Small omnivorous mammal "mouse"	5.7	1 x 0.53	3.62	**2.37**
Intended use	Bare soil (to represent the pre-emergence use in cereals)				
Active substance/product	GLOB1310aH				
Application rate (g/ha)	1 × 900 g a.s./ha (1.5L GLOB1310aH/ha: 810 g Aclonifen/ha +90 g Flufenacet/ha)				
Reprod. toxicity (mg/kg bw/d)	8.58				
TER criterion	5				
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening					
Bare soil	Small granivorous mammal	6.6	1 x 0.53	3.14	**2.72**
First Tier					
Bare soil, BBCH<10	Small omnivorous mammal "mouse"	5.7	1 x 0.53	2.72	**3.16**

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

In addition, a combined risk assessment for effects was performed as well using the following equation and assuming a direct proportionality of the TER to the NOEL:

$$TER(mix) = \left(\sum_i \frac{1}{TER(a.s._i)} \right)^{-1}$$

where:

$TER_{(a.s.i)}$= calculated TER for the active substance i

The TER long-term mix of GLOB1310aH – pre-emergence (2L/ha) for mammals at first step: 1/[(1/2.45)+(1/68.96)]= **2.36**

The TER long-term mix of GLOB1310aH – pre-emergence (1.5L/ha) for mammals at first step: 1/[(1/3.26)+(1/91.95)]= **3.15**

The TER_{lt} value is not higher than the Annex VI trigger value of 5, indicating that GLOB1310aH poses undue chronic risk to mammals following application of 2.0L/ha or 1.5L/ha of GLOB1310aH at pre-emergence to winter cereals. Therefore further considerations are necessary.

Higher tier combined risk assessment

For the highest Tier risk assessment reference is made to the higher tier risk assessment of Aclonifen on the woodmice PT refinements.

Table 0-9: GLOB1310aH: screening assessment and first tier of long-term/reproductive risk for mammals due to the use of GLOB1310aH in cereals at pre-emergence 2L/ha and 1.5L/ha

Intended use	Bare soil (to represent the pre-emergence use in cereals)					
Active substance/product	GLOB1310aH					
Application rate (g/ha)	1 × 1200 g a.s./ha (2L GLOB1310aH/ha: 1080 g Aclonifen/ha +120 g Flufenacet/ha)					
Reprod. toxicity (mg/kg bw/d)	8.58					
TER criterion	5					
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening						
Bare soil	Small granivorous mammal	6.6	1 x 0.53	0.38	1.595	5.37
First Tier						
Bare soil, BBCH<10	Small omnivorous mammal “mouse”	5.7	1 x 0.53	0.38	1.377	6.22
Intended use	Bare soil (to represent the pre-emergence use in cereals)					
Active substance/product	GLOB1310aH					
Application rate (g/ha)	1 × 900 g a.s./ha (1.5L GLOB1310aH/ha: 810 g Aclonifen/ha +90 g Flufenacet/ha)					
Reprod. toxicity (mg/kg bw/d)	8.58					
TER criterion	5					
Crop scenario Growth stage	**Indicator/generic focal species**	**SV_m**	**MAF_m × TWA**	**PT**	**DDD_m (mg/kg bw/d)**	**TER_{lt}**
Screening						
Bare soil	Small granivorous mammal	6.6	1 x 0.53	0.38	1.196	7.17

First Tier						
Bare soil, BBCH<10	Small omnivorous mammal "mouse"	5.7	1 x 0.53	0.38	1.033	8.30

SV: shortcut value; MAF: multiple application factor; TWA: time-weighted average factor; DDD: daily dietary dose; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

In addition, a combined risk assessment for sublethal effects was performed as well using the following equation and assuming a direct proportionality of the TER to the NOEL:

$$TER(mix) = \left(\sum_i \frac{1}{TER(a.s._i)} \right)^{-1}$$

where:

$TER_{(a.s.i)}$= calculated TER for the active substance i

Please note that in the following calculations the PT refinement was applied to AClonifen only and not to Flufenacaet, thus the obtained values can be considered conservative.

The TER long-term mix of GLOB1310aH – pre-emergence (2L/ha) for mammals at first step:
1/[(1/6.45)+(1/68.96)]= 5.89

The TER long-term mix of GLOB1310aH – pre-emergence (1.5L/ha) for mammals at first step:
1/[(1/8.6)+(1/91.95)]= 7.86

The TER_{lt} value is higher than the Annex VI trigger value of 5, indicating that GLOB1310aH poses undue chronic risk to mammals following application of 2.0L/ha or 1.5L/ha of GLOB1310aH at pre-emergence to winter cereals.

9.3.2.4 Drinking water exposure

When necessary, the assessment of the risk for mammals due to uptake of contaminated drinking water is conducted for a small omnivorous mammal with a body weight of 21.7 g (*Apodemus sylvaticus*) and a drinking water uptake rate of 0.24 L/kg bw/d (*cf.* Appendix K of EFSA/2009/1438).

Puddle scenario

Due to the characteristics of the exposure scenario in connection with the standard assumptions for water uptake by animals, no specific calculations of exposure and TER are necessary when the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed 50 in the case of less sorptive substances (Koc < 500 L/kg) or 3000 in the case of more sorptive substances (Koc ≥ 500 L/kg).

Aclonifen

As the Koc exceeds 500 L/kg, Aclonifen belongs to the group of more sorptive substances. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use group 3 also covers the risk for mammals from all other intended uses in groups 1 and 2 (see 0).

Effective application rate (g/ha)	1080			Trigger
Acute toxicity (mg/kg bw)	5000	quotient=	0.22	3000
Reprod. toxicity (mg/kg bw/d)	8	quotient=	135	3000

Since the ratio of effective application rate (in g/ha) to relevant endpoint (in mg/kg bw/d) does not exceed the critical value of 3000, no quantitative risk assessment (calculation of TER values) is necessary.

Flufenacet

Flufenacet with a K(f)oc of 187 mL/g, belongs to the group of less sorptive substances. For the risk assessment the highest intended application rate is used as worst case and it covers all requested uses.

Effective application rate (g/ha)	=	120			Trigger
Acute toxicity (mg/kg bw)	=	589	quotient	=	0.204	50
Reprod. toxicity (mg/kg bw/d)	=	25	quotient	=	4.8	50

The resulting ratios do not exceed the threshold value of 50 for flufenacet, thus a quantitative drinking water risk assessment for the puddle scenario is not triggered.

9.3.2.5 Effects of secondary poisoning

The log P_{ow} of Aclonifen amounts to 4.37 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is therefore required.

The log P_{ow} of Flufenacet amounts to 3.20 and thus exceeds the trigger value of 3. A risk assessment for effects due to secondary poisoning is therefore required.

Risk assessment for earthworm-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for vermivorous mammals is assessed for a small mammal of 10 g body weight with a daily food consumption of 12.8 g. Bioaccumulation in earthworms is estimated based on predicted concentrations in soil.

Aclonifen

Table 0-10: Assessment of the risk for earthworm-eating mammals due to exposure to Aclonifen via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals (2L GLOB1310aH/ha)

Parameter	Aclonifen	comments
$PEC_{21d\ TWA}$ + $PEC_{soil\ plateau}$ (mg/kg soil)	1.9286 (1.3876+0.541)	$PEC_{21d\ TWA}$ + $PEC_{soil\ plateau}$ dRR Part B8 Annex point 8.7.2
log P_{ow} / P_{ow}	4.37 / 23442	EFSA Scientific Report, 2008
Koc	7126	Arithmetic mean ~~Worst case value~~ (n = 3)
foc	0.02	Default
BCF_{worm}	1.97 1.98	$BCF_{worm/soil}$ = ($PEC_{worm,ww}$/$PEC_{soil,dw}$) = (0.84 + 0.012 × P_{ow}) / foc × Koc
PEC_{worm}	3.92 3.81	PEC_{worm} = PEC_{soil} × $BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	5.02 4.88	DDD = PEC_{worm} × 1.28
NOEL (mg/kg bw/d)	8	DE, Addendum 4 to DAR, 2011
TER_{lt}	**1.59** **1.64**	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

Table 0-11: Assessment of the risk for earthworm-eating mammals due to exposure to Aclonifen via bioaccumulation in earthworms (secondary poisoning) for the intended use in cereals (1.5L GLOB1310aH/ha)

Parameter	Aclonifen	comments
$PEC_{21d\ TWA}$ + $PEC_{soil\ plateau}$ (mg/kg soil)	1.447 (1.041+0.406)	$PEC_{21d\ TWA}$ + $PEC_{soil\ plateau}$ dRR Part B8 Annex point 8.7.2
log P_{ow} / P_{ow}	4.37 / 23442	EFSA Scientific Report, 2008
Koc	7126	Arithmetic mean ~~Worst case value~~ (n = 3)
foc	0.02	Default
BCF_{worm}	~~1.97~~ 1.98	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	~~2.86~~ 2.87	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	3.67	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	8	DE, Addendum 4 to DAR, 2011
TER_{lt}	**2.18**	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

According to the risk assessment presented above, an unacceptable risk for earthworm-eating mammals after use of GLOB1310aH is indicated for all intended application rates.

As described in section 9.3.2.2, drilled cereal fields (pre-emergence) are generally unattractive habitats for small mammals (shrew) since these organisms would prefer dense vegetation that offer protection from predator as well as balanced environmental conditions such humidity and temperature. However, the wood mouse was the most abundant species found in pre-emergence like scenarios (Barfknecht 2006, 2010, Funkenhausen and Giessing 2009, Adddendum 4 DAR Aclonifen December 2013) and it is indicated as the representative species for bare soil scenarios in the EFSA Guidance on Birds and Mammals. Additionally, the wood mouse diet includes earthworms (NZ guidance 2020). Moreover, in terms of size and potential exposure, mice already represent the 'worst case' for agricultural areas in Europe's middle zone. Thus, wood mouse can be addressed as an appropriate focal species in the earthworm-eating mammal scenario.

Furthermore, a PT of 0.38 for small mammals in bare soil scenarios is proposed. This value was accepted by the RMS (DE) in Addendum 4 to the DAR Aclonifen and it was derived from studies conducted in maize and sugar beet (please refer to point 9.3.2.2) and it is considered to be a conservative value, since the PT of wood mouse in maize field was 4%. As this refinement was used for the regular long-term risk assessment of aclonifen, it is used the earthworm-eating scenario as well (please refer to 9.3.2.2)

In the EFSA guidance for Birds and Mammals the diet of the wood mouse in bare soils is indicated as follows:

Crop	Scenario	Generic focal species	Representative species	Diet guild	Diet for generic focal species in crops (%)	RUD unit
Bare soil	BBCH<10	Single diet for T1	Wood mouse (*Apodemus sylvicatus*)	Insectivorous	100% ground arthropods	Ground dwelling invertebrates
Bare soil	BBCH<10	Single diet for T1	Wood mouse (*Apodemus sylvicatus*)	Granivorous	100% weed seeds	Small seeds

Bare soil	BBCH<10	Small omnivorous mammal "mouse"	Wood mouse (*Apodemus sylvicatus*)	Omnivorous	50% seed seeds, 50% ground arthropods	Combination (ground invertebrates)

According to the EFSA Guidance on Birds and Mammals, the diet of wood mouse in bare soil may include ground invertebrates (e.g. earthworms). As a worst case, the combined PD indicated in the EFSA Guidance for Birds and Mammals is not included, instead a 100% earthworm diet of the focal species wood mouse are used for the calculations.

In order to correctly apply this refinement a new FIR/bw was calculated according to the appendix G "Calculating exposure for the dietary intake approach" of the EFSA guidance of birds and mammals considering a bw of 21.7g and the following formula:

$$FIR = \left(\frac{DEE}{FE * \left(1 - \frac{MC}{100}\right) * \left(\frac{AE}{100}\right)} \right) \quad \text{[g fresh weight/d]}$$

In which:

DEE = Daily energy expenditure of the indicator species [kJ/d]
FE = Food energy [kJ/dry g]
MC = Moisture content [%]
AE = Assimilation efficiency [%]

The values necessary for the calculations were taken from the table 2 of the appendix G of the aforementioned EFSA guidance. Thus:
DEE= 58.8259 kJ/d (log a: 0.814)
FE= 19.4 kJ/g dry
MC= 84.3%
AE= 87%

Thus the FIR calculated for a wood mouse eating 100% earthworms is 22.20, and then considering a bw of 21.7g the FIR/bw is 1.023

Table 9-3-12: Higher-tier risk assessment, addressing the wood mouse (Apodemus sylvaticus) as earthworm-eating mammal for the intended uses of GLOB1310aH

FIR/bw*	PT**	Diet	PD	PECworm	TWA	DDD	NOEL [mg/(kg bw x d)]	TER = NOEL/ DDD
1.023	0.38	100% earthworms	1	~~3.92~~ 3.81 (calculate for 2L/ha, Table 9.3-10)	-	1.523 1.481	8	~~5.2~~ 5.4
1.023	0.38	100% earthworms	1	~~2.86~~ 2.87 (calculate for 1.5L/ha, Table 9.3-11)	-	1.112 1.116	8	7.2

*FIR/bw as indicated in the Appendix A of the EFSA Guidance Risk Assessment for Birds and Mammals
**PT refinement already accepted by the RMS (DE) in Addendum 4 to the DAR Aclonifen

In summary, for the refinement it is proposed to use wood mouse as the appropriate earthworm-eating

focal species on bare soils (representative for pre-emergence use), including specific portion of time (PT) values and keeping the conservative approach of a diet based on 100% earthworms was applied and it shows that the risk is acceptable.

Flufenacet

Table 0-13: Assessment of the risk for earthworm-eating mammals due to exposure to Flufenacet via bioaccumulation in earthworms (secondary poisoning) for the intended use of GLOB1310aH in cereals (2L/ha GLOB1310aH)

Parameter	Flufenacet	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.1402	dRR Part B8 Annex point 8.7.2
log P_{ow} / P_{ow}	3.2/1585	EC review report
Koc	187	LoEP section B8
foc	0.02	Default
BCF_{worm}	5.31	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.74	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.95	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	25	
TER_{lt}	26.23	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

Table 0-14: Assessment of the risk for earthworm-eating mammals due to exposure to Flufenacet via bioaccumulation in earthworms (secondary poisoning) for the intended use of GLOB1310aH in cereals (1.5L/ha GLOB1310aH)

Parameter	Flufenacet	comments
PEC_{soil} (twa = 21 d) (mg/kg soil)	0.120	dRR Part B8 Annex point 8.7.2
log P_{ow} / P_{ow}	3.2/1585	EC review report
Koc	187	LoEP section B8
foc	0.02	Default
BCF_{worm}	5.31	$BCF_{worm/soil} = (PEC_{worm,ww}/PEC_{soil,dw})$ $= (0.84 + 0.012 \times P_{ow}) / foc \times Koc$
PEC_{worm}	0.64	$PEC_{worm} = PEC_{soil} \times BCF_{worm/soil}$
Daily dietary dose (mg/kg bw/d)	0.82	$DDD = PEC_{worm} \times 1.28$
NOEL (mg/kg bw/d)	25	
TER_{lt}	30.65	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

TER value is above the required trigger of 5. Accordingly the risk to earthworm-eating birds from the use of GLOB1310aH is acceptable.

Combined risk assessment

As shown in point 9.3.2.3, Aclonifen is the driver of the long term toxicity to mammals of GLOB1310aH, while Flufenacet contribution is marginal. Thus, reference is made to the risk assessment for earthworm-

eating mammals of Aclonifen. However, for completeness check the calculations were performed and presented below.

First Tier

Since GLOB1310aH contains 2 active ingredients, a risk assessment for the mixture was performed using the following equation:

$$TER(mix) = \left(\sum_i \frac{1}{TER(a.s._i)} \right)^{-1}$$

where:

$TER_{(a.s.i)}$= calculated TER for the active substance i

At First Tier 2L formulation/ha:

$TER_{mix} = ((1/1.59) + (1/26.23))^{-1} =$ **1.49**

At First Tier 1.5L formulation/ha:

$TER_{mix} = ((1/2.18) + (1/30.65))^{-1} =$ **2.03**

Refinement

As the refinement mentioned above was used for aclonifen and this is a combined risk assessment the same parameters are applied for flufenacet.

Table 9.3-15: Higher-tier risk assessment of flufenacet, addressing the wood mouse (*Apodemus sylvaticus*) as earthworm-eating mammal for the intended uses of GLOB1310aH

FIR/bw*	PT**	Diet	PD	PECworm	TWA	DDD	NOEL [mg/(kg bw x d)]	TER = NOEL/ DDD
1.023	0.38	100% earthworms	1	0.74 (calculated for 2L/ha, Table 9.3-13)	-	0.287	25	86.9
1.023	0.38	100% earthworms	1	0.64 (calculate for 1.5L/ha, Table 9.3-14)	-	0.248	25	100.5

*FIR/bw as indicated in the Appendix A of the EFSA Guidance Risk Assessment for Birds and Mammals
**PT refinement already accepted by the RMS (DE) in Addendum 4 to the DAR Aclonifen
***Mean RUD for 100% earthworm diet, indicated in the Appendix A of the EFSA Guidance Risk Assessment for Birds and Mammals

The TER values are higher than the trigger indicating an acceptable risk to earthworm eating mammals.

An additional combined risk assessment is presented below.

$$TER(mix) = \left(\sum_i \frac{1}{TER(a.s._i)} \right)^{-1}$$

where:

$TER_{(a.s.i)}$= calculated TER for the active substance i

2L formulation/ha:

$TER_{mix} = ((1/5.2) + (1/86.9))^{-1} = 4.9$

1.5L formulation/ha:

$TER_{mix} = ((1/7.2) + (1/100.5))^{-1} = 6.7$

The TER values are higher than the trigger indicating an acceptable risk to earthworm eating mammals. It is seen that for the dose of 2L/ha the TERlt for the combined toxicity it is slightly below the trigger if 5, namely 4.9. However, the risk assessment calculations were done under a very conservative approach, this is considering a diet of 100% earthworms for the focal species wood mouse. This is not realistic as the wood mouse is an opportunistic feeder. In the Northern Zone Guidance (2020) it is clearly seen that the wood mouse feed from different sources, like arthropods, earthworms, plant seeds, vegetative tissue, etc., and this is variable depending the time of year. For the intended application time of GLOB1310aH, the percentage of the wood mouse diet consisting of earthworms is 13%, 30% and 3% in September, October and November, respectively. When regard as a whole period, from September to December wood mouse can feed on arthropods (assuming earthworms are part of this group) up to 16%. It is acknowledge that the Northern Guidance may be of no relevance for the Central Zone. Nevertheless, it has to be highlighted that the studies summarised in the Northern Zone Guidance were performed in countries like UK and Germany which possess environmental conditions representative of the Central Zone. Moreover, in the EFSA Guidance for birds and mammals, which is not specific to any zone, it is mentioned that wood mouse is a small omnivorous mammal, which diet is based on 50% seeds and 50% ground arthropods (ground invertebrates). It is clear that the wood mouse's diet is not based in one single good, its diet is not 100% based on earthworms. Thus, the TER of 4.9 obtained under the worst case assumption that wood mouse diet is only earthworm-based is very worst case and the risk to earth-worm eating mammals is still acceptable.

Risk assessment for fish-eating mammals via secondary poisoning

According to EFSA/2009/1438, the risk for piscivorous mammals is assessed for a mammal of 3000 g body weight with a daily food consumption of 425 g. Bioaccumulation in fish is estimated based on predicted concentrations in surface water.

Aclonifen

As indicated in the working document on Risk Assessment of Plant Protection Products in the Central Zone, version 1.0 May 2021 it is possible to apply the lowest RAC, instead of the PECsw. However, this approach is only applicable if an acceptable risk for aquatic organisms could be demonstrated for this value. Hence, a risk assessment is presented below where the lowest RAC (i.e. 0.5 µg/L, derived from a chronic fish study) is applied. This refinement is only applicable in compliance with the mitigation measures indicated in the risk assessment for aquatic organisms (point 9.5.2).

Table 0-16: Assessment of the risk for fish-eating mammals due to exposure to Aclonifen via bioaccumulation in fish (secondary poisoning) for the intended use in cereals (2 and 1.5 L/ha)

Parameter	Aclonifen	Comments
RAC (mg/L)	0.0005	Lowest available RAC from the aquatic section (chronic toxicity to fish; NOECgrowth = 0.005 mg a.s./L; AF = 10; EFSA Conclusion 2008)
BCF_{fish}	2896	EFSA Conclusion, 2008
PEC_{fish}	1.45	$PEC_{fish} = PEC_{water} \times BCF_{fish}$

Daily dietary dose (mg/kg bw/d)	0.21	DDD = PEC_{fish} × 0.142
NOEL (mg/kg bw/d)	8	EFSA Conclusion, 2008
TER_{lt}	38.91	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

Additionally, the risk assessment was conducted using the PECsw 21d.

Table 0-17: Assessment of the risk for fish-eating mammals due to exposure to Aclonifen via bioaccumulation in fish (secondary poisoning) for the intended use in cereals (2 L/ha)

Parameter	Aclonifen	comments
PEC_{sw} (max) (mg/L)	0.003486	dRR Part B8 Annex point 8.9.2 Overall highest PECsw twa=21days FOCUS Step 3 (3.486µ/L D1 dicth)
BCF_{fish}	2896	EFSA, 2008
PEC_{fish}	10.09	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	1.43	DDD = PEC_{fish} × 0.142
NOEL (mg/kg bw/d)	8	DE, Addendum 4 to DAR, 2011
TER_{lt}	5.58	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

Table 0-18: Assessment of the risk for fish-eating mammals due to exposure to Aclonifen via bioaccumulation in fish (secondary poisoning) for the intended use in cereals (1.5 L/ha)

Parameter	Aclonifen	comments
PEC_{sw} (max) (mg/L)	0.002598	dRR Part B8 Annex point 8.9.2 Overall highest PECsw twa=21days FOCUS Step 3 (2.5980µ/L D1 dicth)
BCF_{fish}	2896	EFSA, 2008
PEC_{fish}	7.52	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	1.07	DDD = PEC_{fish} × 0.142
NOEL (mg/kg bw/d)	8	DE, Addendum 4 to DAR, 2011
TER_{lt}	7.48	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

Flufenacet

As indicated in the working document on Risk Assessment of Plant Protection Products in the Central Zone, version 1.0 May 2021 it is possible to apply the lowest RAC, instead of the PECsw. However, this approach is only applicable if an acceptable risk for aquatic organisms could be demonstrated for this value. Hence, a risk assessment is presented below where the lowest RAC (i.e. 0.243 µg/L, derived from an acute Lemna study) is applied. This refinement is only applicable in compliance with the mitigation measures indicated in the risk assessment for aquatic organisms (point 9.5.2).

Table 0-19: Assessment of the risk for fish-eating mammals due to exposure to Flufenacet via bioaccumulation in fish (secondary poisoning) for the intended use in cereals (2L/ha)

Parameter	Flufenacet	comments
RAC flufenacet aquatic organisms (mg/L)	0.000243	RAC flufenacet aquatic organisms (mg/L), aquatic macrophytes
BCF_{fish}	71.4	Review report, 2003
PEC_{fish}	~~0.0357~~ 0.0174	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	~~0.005~~ 0.0025	$DDD = PEC_{fish} \times$ ~~0.159~~0.142
NOEL (mg/kg bw/d)	25	EFSA, 2008
TER_{lt}	~~4931.5~~ 10000	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

Additionally, the risk assessment was conducted using the PECsw 21d.

Table 0-20: Assessment of the risk for fish-eating mammals due to exposure to Flufenacet via bioaccumulation in fish (secondary poisoning) for the intended use of GLOB1310aH in cereals 2.0L/ha

Parameter	Flufenacet	comments
PEC_{sw} (twa = 21 d) (mg/L)	0.00348	dRR Part B8 Annex point 8.9.2 Overall highest PECsw twa=21days FOCUS Step 3 (3.48µ/L D2 dicth)
BCF_{fish}	71.4	EC review report
PEC_{fish}	0.248	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.035	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	25	DE, Addendum 4 to DAR, 2011
TER_{lt}	708.5	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

Table 0-21: Assessment of the risk for fish-eating mammals due to exposure to Flufenacet via bioaccumulation in fish (secondary poisoning) for the intended use of GLOB1310aH in cereals 1.5L/ha

Parameter	Flufenacet	comments
PEC_{sw} (twa = 21 d) (mg/L)	0.002258	dRR Part B8 Annex point 8.9.2 Overall highest PECsw twa=21days FOCUS Step 3 (2.258µ/L D1 dicth and R4 stream)
BCF_{fish}	71.4	EC review report
PEC_{fish}	0.161	$PEC_{fish} = PEC_{water} \times BCF_{fish}$
Daily dietary dose (mg/kg bw/d)	0.023	$DDD = PEC_{fish} \times 0.142$
NOEL (mg/kg bw/d)	25	DE, Addendum 4 to DAR, 2011
TER_{lt}	1092	TER criterion = 5

TER values shown in bold fall below the relevant trigger.

All TER values are above the required trigger of 5. Accordingly the risk to fish-eating mammals from the use of GLOB1310aH is acceptable.

Combined risk assessment

As shown in point 9.3.2.3, Aclonifen is the driver of the long term toxicity to mammals of GLOB1310aH, while Flufenacet contribution is marginal. Thus, reference is made to the risk assessment for earthworm-eating mammals of Aclonifen. However, for completeness check the calculations were performed and presented below.

A risk assessment for the mixture was performed using the following equation:

$$TER(mix) = \left(\sum_i \frac{1}{TER(a.s._i)} \right)^{-1}$$

where:

$TER_{(a.s.i)}$= calculated TER for the active substance i

2L formulation/ha:

$TER_{mix} = ((1/5.58) + (1/708.5))^{-1} = 5.53$

1.5L formulation/ha:

$TER_{mix} = ((1/7.48) + (1/1092))^{-1} = 7.43$

9.3.2.6 Biomagnification in terrestrial food chains

Aclonifen

Not relevant.

The results of the ADME studies indicate that Aclonifen has a low bioaccumulation potential. The List of Endpoints of mammalian toxicology states: “Potential for bioaccumulation: no evidence of accumulation”. It is therefore assumed that there will be no biomagnification along the food chain.

Flufenacet

Low potential for accumulation in animal tissue was concluded in the EU review of flufenacet. Since the bioaccumulation potential of the active substance is low no further assessment on biomagnification is required.

9.3.3 Risk assessment for baits, pellets, granules, prills or treated seed

Not relevant. GLOB1310aH is intended for use as a foliar spray.

9.3.4 Overall conclusions

For the two active substances (Aclonifen and Flufenacet), the TER values calculated for recommended scenarios, exceed the trigger values of 10 for acute risk and 5 for long-term risk indicating acceptable risk following the uses indicated in the GAP of the product GLOB1310aH.
Therefore, treatment with GLOB1310aH in accordance with the proposed GAP in winter cereals poses an

acceptable risk to terrestrial vertebrates other than birds.

Review comments:

The risk assessment to mammals was performed in accordance with the recommendation of Guidance Document on Risk Assessment for Birds & Mammals on request from EFSA (EFSA Journal 2009; 7(12):1438).
The results of the 'screening phase' acute dietary risk assessment - Toxicity Exposure Ratios (TER_A) were calculated taking into account the EU agreed endpoints for most sensitive species for the active substance and using the EFSA Bird and Mammal risk assessment calculator for the higher predicted application rate than it is foreseen in GAP exceeding the trigger set by Commission regulation (EU) 546/2011 for accept-ability of effects. Revealed that there is no potential of risk for wild mammals resulting from acute exposure to active substances following use of GLOB1310aH in compliance with proposed GAP.

The results of the 'screening phase' long term dietary risk assessment - Toxicity Exposure Ratios (TER_{LT}) were calculated taking into account the EU agreed endpoints for most sensitive species for the active substance and using the EFSA Bird and Mammal risk assessment calculator for the higher predicted application rate than it is foreseen in GAP not exceeding the trigger set by Commission regulation (EU) 546/2011 for accept-ability of effects. Revealed that there is potential of risk for wild mammals resulting from long-term exposure to active substance Aclonifen following use of GLOB1310aH in compliance with proposed GAP.

Higher tier risk assessment therefore is proposed by applicant based on refinement of proportion of active time (PT). PT of 38% obtained in sugarbeet is used as a surrogate for winter cereals knowing that this is significantly overestimating the real portion of time spend by woodmice in drilled fields. This proposal is considered acceptable by the zRMS, PT of 0.38 was accepted by the RMS Germany (DE, Addendum 4 to DAR, 2011). Therefore based on refinement PT value an acceptable chronic risk can be concluded.

A quantitative drinking water risk assessment is not triggered for the proposed use pattern of Glosset Ace (GLOB1310aH) according to EFSA/2009/1438 criteria and therefore the risk to mammals via drinking water is acceptable.

The risk to mammals from consuming contaminated earthworms was shown to be acceptable for flufenacet. For aclonifen TER values do not meet the trigger value (5). For aclonifen the applicant proposed a refined long-term DDD and TER calculation for earthworm eating mammals. It mainly relies on the refinement of PT (0.4 instead of 1) and a diet based on 100% earthworms. The acceptability of the assessment for aclonifen should be confirmed on member state level.

The risk to mammals from consuming contaminated fish was shown to be acceptable for both active substances.

All intended uses of Glosset Ace (GLOB1310aH) are causing an unacceptable risk for the chronic risk assessment of mammals in the first tier in case of the a.s. aclonifen. Therefore, the mixture toxicity assessment for long-term effects is consequently also indicating an unacceptable risk (considering Tier 1 data). The applicant has provided higher Tier refinements for aclonifen and an acceptable risk regarding mixture toxicity can be concluded.

No risk mitigation measures are required.

Conclusion
According to the performed risk assessment there is no potential of risk to mammals resulting from exposure to active substance following use of Glosset Ace (GLOB1310aH) in compliance with proposed GAP.

9.4 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians) (KCP 10.1.3)

Not relevant.

9.5 Effects on aquatic organisms (KCP 10.2)

9.5.1 Toxicity data

Studies on the toxicity to aquatic organisms have been carried out with aclonifen, flufenacet and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on aquatic organisms of GLOB1310aH were not evaluated as part of the EU assessment of aclonifen and flufenacet. New data submitted with this application are listed in 0 and summarised in 0.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 0-1: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Aclonifen, flufenacet and relevant metabolites

Species	Substance	Exposure System	Results	Reference
Fish-acute				
Oncorhynchus mykiss	aclonifen	96 h, s	LC_{50} > **0.67** mg a.s./L $_{mm}$	EFSA 2008
Lepomis macrochirus	Flufenacet	96h, ss	LC_{50} = **2.13** mg a.s./L $_{mm}$	SANCO/7469/VI/98-Final, 03 July 2003
Oncorhynchus mykiss	FOE-sulfonic acid	96h, s	LC_{50} = **86.7** mg metab/L $_{mm}$	SANCO/7469/VI/98-Final, 03 July 2003
Oncorhynchus mykiss	FOE-oxalate	Acute	LC_{50}/EC_{50} **86.7** mg metab/L**	-
Oncorhynchus mykiss	Thiadone	96h, s	LC_{50} = **9.1** mg metab/L $_{mm}$	SANCO/7469/VI/98-Final, 03 July 2003
Oncorhynchus mykiss	FOE-methylsulfide	96h, s	LC_{50} = **86.7**** mg metab/L $_{mm}$	-
Fish-Chronic				

Species	Substance	Exposure System	Results	Reference
Pimephales promelas	Aclonifen	35 d, ELS, flow-thorugh	NOECgrowth = **0.005** mg a.s./L $_{mm}$ NOEChatch = 0.011 mg a.s./L $_{mm}$	EFSA 2008
Oncorhynchus mykiss	Flufenacet	97d, ELS, f	NOEC = **0.2** mg a.s./L	SANCO/7469/VI/98-Final, 03 July 2003
Aquatic invertebrates-Acute				
*Daphnia magna**	Aclonifen	48 h, s	EC_{50} = **1.2** mg a.s./L $_{mom}$ (0.952 mg a.s./L mm)	EFSA 2008
Daphnia magna	Flufenacet	48h, s	LC_{50} = **30.9** mg a.s./L $_{mm}$	SANCO/7469/VI/98-Final, 03 July 2003
Daphnia magna	FOE-sulfonic acid	48h, s	LC_{50} >**87.3** mg metab/L $_{mm}$	SANCO/7469/VI/98-Final, 03 July 2003
Daphnia magna	FOE-oxalate	48h, s	LC_{50} >**87.3**** mg metab/L $_{mm}$	-
Daphnia magna	Thiadone	48h, s	LC_{50} = **31.7** mg metab/L $_{mm}$	SANCO/7469/VI/98-Final, 03 July 2003
Daphnia magna	FOE-methylsulfide	48h, s	LC_{50} > **87.3** mg met/L**	-
Aquatic invertebrates-Chronic				
Daphnia magna	Aclonifen	21 d, ss	NOEC = **0.016** mg a.s./L $_{mm}$	EFSA 2008
Daphnia magna	Flufenacet	21d, ss	NOEC = **3.26** mg a.s./L $_{mm}$	SANCO/7469/VI/98-Final, 03 July 2003
Sediment dwelling organisms				
Chironomus riparius	Aclonifen	28 d, spiked water	NOEC = 0.472 mg a.s./L $_{nom}$	EFSA 2008
Chironomus riparius	Aclonifen	28 d, spiked sediment	NOEC = 32 mg a.s./kg sediment (dw) nom	EFSA 2008
Algae				
*Desmodesmus subspicatus****	Aclonifen	96h, s	E_bC_{50} = 0.0067 mg a.i./L E_rC_{50} = 0.0069 mg a.i./L	EFSA, 2008 DE, Addendum 4 to DAR, 2011
*Navicula pelliculosa****	Aclonifen	72h, s	E_bC_{50} = 0.47 mg a.i./L E_rC_{50} = 1.2 mg a.i./L	EFSA, 2008 DE, Addendum 4 to DAR, 2011
*Chlorella vulgaris****	Aclonifen	72h, s	E_bC_{50} = 0.0866 mg a.i./L E_rC_{50} = 0.450 mg a.i./L	DE, Addendum 4 to DAR, 2011

Species	Substance	Exposure System	Results	Reference
*Chlamydomonas reinhardii****	Aclonifen	72h, s	E_bC_{50} = 0.0158 mg a.i./L E_rC_{50} = 0.0753 mg a.i./L	DE, Addendum 4 to DAR, 2011
*Xanthonema debile****	Aclonifen	72h, s	E_bC_{50} = 0.0987 mg a.i./L E_rC_{50} = 0.319 mg a.i./L	DE, Addendum 4 to DAR, 2011
*Closterium cornu****	Aclonifen	72h, s	E_bC_{50} = 0.0682 mg a.i./L E_rC_{50} = 0.112 mg a.i./L	DE, Addendum 4 to DAR, 2011
*Synechococcus leopoliensis****	Aclonifen	72h, s	E_bC_{50} = 0.037 mg a.i./L E_rC_{50} = 0.0749 mg a.i./L	DE, Addendum 4 to DAR, 2011
*Nannochloropsis limnetica****	Aclonifen	72h, s	E_bC_{50} = 0.461 mg a.i./L E_rC_{50} = 0.513 mg a.i./L	DE, Addendum 4 to DAR, 2011
HC5***	Aclonifen	-	HC5=9.9 µg a.s./L	DE, Addendum 4 to DAR, 2011
HC5****	Aclonifen	-	HC5= **5,3** µg a.s./L	DE, Addendum 4 to DAR, 2011
Pseudokirchneriella subcapitata	Flufenacet	72 h, s	E_bC_{50} = 0.00204 mg a.i./L E_rC_{50} = **0.0045** mg a.i./L	SANCO/7469/VI/98-Final, 03 July 2003
Scenedesmus subspicatus	FOE-sulfonic acid	120h	EC_{50} >**86.7** mg metab/L**	SANCO/7469/VI/98-Final, 03 July 2003
Scenedesmus subspicatus	FOE-oxalate	120h	EC_{50} >**86.7** † mg metab/L	SANCO/7469/VI/98-Final, 03 July 2003
S. capricornutum	Thiadone	72h	EbC_{50} =4.1 mg metab/L ErC_{50} =**15.0** mg metab/L	SANCO/7469/VI/98-Final, 03 July 2003 Addendum (April 2000)
S. capricornutum	FOE-methylsulfide	72h, s	EC_{50} =83.3 mg metab/L	SANCO/7469/VI/98-Final, 03 July 2003
Aquatic plants				
Lemna gibba	Aclonifen	14 d, s	E_bC_{50} = 0.006 mg a.i./L E_rC_{50} = **0.012** mg a.i./L	EFSA 2008
Lemna gibba	Flufenacet	7d	EC_{50} =**0.00243** mg a.i./L nom	SANCO/7469/VI/98-Final, 03 July 2003
Lemna gibba	FOE-sulfonic acid	14d, s	EC_{50} > 86.7 mg a.i./L	SANCO/7469/VI/98-

Species	Substance	Exposure System	Results	Reference
				Final, 03 July 2003
Lemna gibba	FOE-oxalate	7d	EC_{50} >**86.7** mg met/L**	-
Lemna gibba	FOE-methylsulfide	7d	EC_{50} >**86.7** mg met/L**	-
Higher-tier studies (micro- or mesocosm studies)				
Microcosm (macrophytes and periphyton)	Flufenacet (Flufenacet WG 60)	84d, s	NOEC = **0.012** mg a.i./L	SANCO/7469/VI/98-Final, 03 July 2003

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations; im: based on initial measured concentrations

*Since the measured concentrations are between 83 and 94 % of the nominal concentrations during the course of the test, the EC50 calculation based on nominal concentrations is considered to be appropriate. Therefore, the EC50 of 1.2 mg/L is used for the risk assessment.

** No data available for this metabolite, therefore based on the molecular structure the assumption is made that the toxicity of FOE-oxalate and FOE-sulfonic acid as well as the toxicity of FOE-sulfonic acid and FOE-methylsulfide is comparable and the endpoints for FOE-sulfonic acid are used for FOE-oxalate and FOE-methylsulfide. Moreover, this is confirmed by the similar endpoints for algae of FOE-sulfonic acid and FOE-methylsulfide.

zRMS comments: the endpoints relevant to the risk assessment for the following flufenacet metabolites have been established at EU level (SANCO/7469/VI/98-Final, 03 July 2003):
- fish: FOE-sulfonic acid, Thiadone
- invertebrates: FOE-sulfonic acid, Thiadone,
- algae: FOE-sulfonic acid, Thiadone, FOE-methylsulfide,
- aquatic plants: FOE-sulfonic acid,

and only these endpoint were considered in the risk assessment by zRMS.

***With reference to the Confirmatory data for Aclonifen (DE, Addendum 4 to DAR, 2011), a HC5 based on ErC50 values for 8 species was calculated (i.e. 9.9 µg/L). As commented by ANSES in the pre-submission form, for primary producers, the recommendations in EFSA Aquatic guidance (EFSA Journal 2013;11(7):3290, 268 pp) are to calculate the SSD-RAC both on the basis of the median HC5 and the application of an assessment factor of 3. Therefore, the risk assessment is performed using a RAC of 3.3 µg/L (9.9/3).

**** With reference to the Confirmatory data for Aclonifen (DE, Addendum 4 to DAR, 2011), a HC5 based on EbC50 values was recalculated by zRMS. The value of HC5 = 5.3 µg/L with the assessment factor of 5 should be used in the risk assessment.

Table 0-2: Endpoints and effect values relevant for the risk assessment for aquatic organisms – GLOB1310aH

Species	Substance	Exposure System	Results	Reference
Daphnia magna	GLOB1310aH	48 h, s	EC_{50} = **6.12** mg formulation/L $_{nom}$ (equivalent to 2.71 mg/L Aclonifen and 0.307mg/L Flufenacet, total **3.017** µg a.s./L)	Juckeland D., 2021 Study report 21 48 ADL 0001 (and amendment 1)
Pseudokirchneriella	GLOB1310aH	72 h, s	E_rC_{50} = **54.2** µg	Juckeland D., 2021

Species	Substance	Exposure System	Results	Reference
subcapitata			formulation/L mm (equivalent to 23.7 µg Aclonifen/L_{mm} + 2.92 µg Flufenacet/$L_{nom,}$ total **26.62** µg a.s./L) E_yC_{50} = 16.0 µg formulation/L_{mm} (equivalent to 6.88 µg Aclonifen/L_{mm} + 0.98 µg Flufenacet/$L_{nom,}$ total 7.86 µg a.s./L)	Study report 21 48 AAL 0001
Desmodesmus subspicatus	GLOB1310aH	72 h, s	E_rC_{50} = **103.8** µg formulation/L_{mm} (equivalent to 44.82 µg Aclonifen/L_{mm} + 6.28 µg Flufenacet/$L_{nom,}$ total **51.1** µg a.s./L) E_yC_{50} = 37.4 µg formulation/L_{mm} (equivalent to 16.0 µg Aclonifen/L_{mm} + 2.39 µg Flufenacet/$L_{mm,}$ total 18.39 µg a.s./L)	Juckeland D., 2021 Study report 21 48 AAL 0017
Lemna gibba	GLOB1310aH	7d, s	$E_rC_{50\ frond}$ = 11.6 µg formulation/L_{mm} (equivalent to 4.96 µg Aclonifen/L_{mm} + 0.74 µg Flufenacet/$L_{mm,}$ total 5.7 µg a.s./L) $E_rC_{50\ biomass}$ = **3.62**µg formulation/L_{mm} (equivalent to 1.55 µg Aclonifen/L_{mm} + 0.24 µg Flufenacet/$L_{mm,}$ total **3.86** µg a.s./L)	Juckeland D., 2021 Study report 21 48 ALE 0001
Myriophyllum spicatum	GLOB1310aH		$E_rC_{50\ shoot\ lenght}$ = **301.9** µg formulation/L_{mm} (equivalent to 132.5 µg Aclonifen/L_{mm} + 16.2 µg Flufenacet/$L_{nom,}$ total **148.7** µg a.s./L)	Juckeland D., 2021 Study report 21 48 AMS 0001

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentrations

9.5.1.1 Justification for new endpoints

Studies on the formulation were done for aquatic invertebrates, algae and aquatic plants, but not for fish in order to avoid studies conducted on vertebrates. On an acute toxicity point of view, for both active Aclonifen and Flufenacet, the acute fish endpoints are much higher (670 µg/L in Aclonifen and 2130 µg/L for Flufenacet) than the endpoints of algae (~~9.9~~ 5,3 µg/L for aclonifen (from HC5 approach) and 12 µg/L (from microcosms study) for flufenacet) and the one of Lemna (12 µg/L for aclonifen and 12 µg/L

(from microcosms study) for flufenacet). So, at the acute level the fish is not expected to be more sensitive than algae and macrophytes.

However, considering of the chronic toxicity, the risk assessment of Aclonifen is driven by the lowest fish endpoint (i.e. $NOEC_{growth}$ = 5 µg/L). According to the data requirements for product evaluations, formulation studies on the long-term toxicity are only triggered when '*it is not possible to extrapolate from data obtained in the corresponding studies on the active substance (for example the plant protection product is more acutely toxic than the active substance as manufactured by a factor of 10)*' (Commission Regulation (EU) No 284/2013). In the current situation, the toxicity of the formulated product GLOB1310aH is not higher than the one of the active substances. In addition, it is generally observed that chronic effects are mainly driven by the active ingredient (and not the co-formulant), as the product is quickly under its active ingredient form in the environment. We also refer to the analysis of the predicted toxicity versus observed toxicity (below), that did not show any synergistic effect of both active ingredients in the formulation GLOB1310aH on algae, Daphnia and macrophytes). From the combined risk assessment it is clear that the toxicity driver of the toxicity to fish is Aclonifen. Therefore, the applicant considers that the risk assessment is covered by the respective risk assessment of each active ingredient for chronic fish and that a vertebrate's study on fish was not necessary since it would not have change the outcome of risk assessment.

For flufenacet, a microcosm experiment (macrophyte, duckweed and periphyton) with Flufenacet WG 60, which is reported in detail in the addendum to the DAR (Foekema E.M. and Jak R.G., 1999), did not show significant treatment related effects at any treatment level (0.75, 1.5, 3, 6, 12 and 24 µ as/L). Nevertheless, some slight differences in community metabolism (O2 and pH) were noted in the highest treatment level (24 µ as/L) as was a slightly reduced growth of some macrophytes and periphyton. All other measured parameters were unaffected. All the observations at the highest treatment level were slight and transient only, with a recovery before the end of the study. The fact that treatment related effects were only observed at the highest concentration as well as the observed recovery of even the most sensitive endpoints (community metabolism) is in accordance with the short half-life of flufenacet in the water column. In the study a NOEC of 12 µg/l was established.

According the "Guidance on tiered risk assessment for edge-of-field surface waters, EFSA, 2013", the NOEC of 12 µg/L for the microcosm study can be considered as "Effect class 1" as no treatment-related effects demonstrated for the most sensitive endpoints, no (statistically and/or ecologically significant) effects observed as a result of the treatment and the observed differences between treatment and controls show no clear causal relationship. For the Effect class 1 concentration it is recommended an Assessment factor for ETO-RACsw;ac derivation (ecological threshold option) of 2. For risk assessment proposal a RAC of 4 µg/L considering an AF of 5 instead 2 is proposed as a conservative worst case.

9.5.2 Risk assessment

The evaluation of the risk for aquatic and sediment-dwelling organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).
The relevant global maximum FOCUS Step 1, 2 and 3 PEC_{SW} for risk assessments covering the proposed use pattern and the resulting PEC/RAC ratios are presented in the table below.

In the following table, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

9.5.2.1 Aclonifen

In the following table, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}, PEC_{SED}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given per intended use for each FOCUS scenario and each organism group.

Table 0-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aclonifen for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of 1.5L GLOB1310aH/ha in winter cereals at pre-emergence (810g Aclonifen/ha pre-emergence)

Group		**Fish acute**	**Fish prolonged**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic macrophytes**	**Sed. dwell. prolonged**		**Sed. dwell. prolonged**
Test species		*O. mykiss*	*P. promelas*	*P. promelas*	*D. magna*	*D. magna*	*HC5*	*Lemna gibba*	*C. riparius*		*C. riparius*
Endpoint (µg/L)		LC_{50}	NOEC growth	NOEC hatching	LC_{50} nom	NOEC	EC_{50}	ErC_{50}	NOEC		NOEC
		670	5	11	1200	16	~~9.9~~/5.3	12	472		~~3200~~ 32000
AF		100	10	10	100	10	~~3~~ 5	10	10		10
RAC (µg/L)		6.7	0.5	1.1	12	1.6	~~3.3~~ 1,06	1.2	47.2		~~320~~ 3200
FOCUS Scenario	**PEC max (µg/L)**									**PEC max (µg/L)**	
Step 1											
Worst-case	40.8211	**6.092**	**81.642**	**37.11**	**3.401**	**25.513**	**~~12.37~~ 38.510**	**34.017**	0.864853814	1770	**~~5.531~~** 0.553
Step 2											
N-Europe	17.1896	**2.565**	**34.379**	**15.626**	**1.432**	**10.743**	**~~5.208~~ 16.217**	**14.324**	0.364186441	895.0279	**~~2.796~~** 0.280
S-Europe	13.9977	**2.089**	**27.99**	**12.72**	**1.16**	**8.748**	**~~4.241~~ 13.205**	**11.664**	0.296561441	725.3994	**~~2.266~~** 0.227
Step 3									0		
D1 (ditch)	5.155	0.769	**10.310**	**4.686**	0.430	**3.222**	**~~1.562~~ 4.863**	**4.296**	0.109	19.190	~~0.060~~ 0.006
D1 (stream)	6.076	0.907	**12.152**	**5.524**	0.506	**3.798**	**~~1.841~~ 5.732**	**5.063**	0.129	2.742	~~0.009~~ 0.0009
D2 (ditch)	5.16	0.770	**10.320**	**4.691**	0.430	**3.225**	**~~1.564~~ 4.868**	**4.300**	0.109	16.180	~~0.051~~ 0.005
D2 (stream)	6.187	0.923	**12.374**	**5.625**	0.516	**3.867**	**~~1.875~~ 5.837**	**5.156**	0.131	14.340	~~0.045~~ 0.004
D3 (ditch)	5.082	0.759	**10.164**	**4.620**	0.424	**3.176**	**~~1.540~~ 4.794**	**4.235**	0.108	2.826	~~0.009~~ 0.0009
D4 (pond)	0.2625	0.039	0.525	0.239	0.022	0.164	~~0.080~~ **0.248**	0.219	0.006	1.155	~~0.004~~ 0.0005
D4 (stream)	5.937	0.886	**11.874**	**5.397**	0.495	**3.711**	**~~1.799~~ 5.601**	**4.948**	0.126	0.942	~~0.003~~ 0.0003
D5 (pond)	0.2625	0.039	0.525	0.239	0.022	0.164	~~0.080~~ 0.248	0.219	0.006	1.290	~~0.004~~ 0.0004
D5 (stream)	6.406	0.956	**12.812**	**5.824**	0.534	**4.004**	**~~1.941~~ 6.043**	**5.338**	0.136	1.327	~~0.004~~ 0.0004
D6 (ditch)	5.138	0.767	**10.276**	**4.671**	0.428	**3.211**	**~~1.557~~ 4.847**	**4.282**	0.109	13.910	~~0.043~~ 0.0043
R1 (pond)	0.2928	0.044	0.586	0.266	0.024	0.183	~~0.089~~ 0.276	0.244	0.006	2.757	~~0.009~~ 0.0009

R1 (stream)	4.512	0.673	**9.024**	**4.102**	0.376	**2.820**	**~~1.367~~ 4.257**	**3.760**	0.096	8.272	0.026
R3 (stream)	6.266	0.935	**12.532**	**5.696**	0.522	**3.916**	**~~1.899~~ 5.911**	**5.222**	0.133	283.600	0.886
R4 (stream)	4.54	0.678	**9.080**	**4.127**	0.378	**2.838**	**~~1.376~~ 4.283**	**3.783**	0.096	5.592	0.017

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 0-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aclonifen for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of 2.0L GLOB1310aH/ha in winter cereals at pre-emergence (1080g Aclonifen/ha)

Group		**Fish acute**	**Fish prolonged**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic macrophytes**	**Sed. dwell. prolonged**		**Sed. dwell. prolonged**
Test species		*O. mykiss*	*P. promelas*	*P. promelas*	*D. magna*	*D. magna*	*HC5*	*Lemna gibba*	*C. riparius*		*C. riparius*
Endpoint (µg/L)		LC_{50}	NOEC growth	NOEC hatching	LC_{50} nom	NOEC	EC_{50}	ErC_{50}	NOEC		NOEC
		670	5	11	1200	16	~~9.9~~ 5.3	12	472		3200
AF		100	10	10	100	10	~~3~~ 5	10	10		10
RAC (µg/L)		6.7	0.5	1.1	12	1.6	~~3.3~~ 1.06	1.2	47.2		~~320~~ 3200
FOCUS Scenario	**PEC max (µg/L)**									**PEC max (µg/L)**	
Step 1											
Worst-case	54.4281	**8.1235**	**108.8**	**49.4800**	**4.535**	**34.017**	**~~16.49~~ 51.347**	**45.35**	**1.153**	2370	**~~7.406~~** 0.741
Step 2							-				
N-Europe	22.9194	**3.420**	**45.838**	**20.83**	**1.909**	**14.324**	**~~6.945~~ 21.622**	**19.0995**	0.4855	1190	**~~3.718~~** 0.372
S-Europe	18.6636	**2.7856**	**37.327**	**16.966**	**1.555**	**11.664**	**~~5.655~~ 17.607**	**15.553**	0.3954	967.2	**~~3.0225~~** 0.302
Step 3							-				
D1 (ditch)	6.878	**1.02656**	**13.756**	**6.2527**	0.573	**4.2987**	**~~2.0842~~ 6.489**	**5.7316**	0.1457	~~25.61~~ 33.0966	~~0.080~~ 0.0103
D1 (stream)	8.106	**1.2098**	**16.212**	**7.3690**	0.6755	**5.0662**	**~~2.4563~~ 7.647**	**6.755**	0.1717	~~4.929~~ 6.3699	~~0.0154~~ 0.0019
D2 (ditch)	6.884	**1.02746**	**13.768**	**6.258**	0.5736	**4.3025**	**~~2.0860~~ 6.494**	**5.7366**	0.1458	~~21.59~~ 26.2887	~~0.067~~ 0.0082
D2 (stream)	8.254	**1.2319**	**16.508**	**7.5036**	0.6878	**5.1587**	**~~2.5012~~**	**6.8783**	0.1748	~~25.78~~	~~0.0805~~

							7.787			31.3906	0.0098
D3 (ditch)	6.78	**1.0119**	**13.56**	**6.1636**	0.565	**4.237**	~~**2.0545**~~ **6.396**	**5.65**	0.1436	~~3.769~~ 4.3859	~~0.011~~ 0.0014
D4 (pond)	0.3502	0.0522	0.7004	0.3183	0.0291	0.2188	~~0.1061~~ 0.330	0.291	0.0074	~~2.309~~ 2.8756	~~0.007~~ 0.0009
D4 (stream)	7.921	**1.182**	**15.842**	**7.2009**	**0.6600**	**4.950**	~~**2.4003**~~ **7.473**	**6.600**	0.167	~~1.692~~ 2.1072	~~0.0052~~ 0.0007
D5 (pond)	0.3503	0.052	0.7006	0.3184	0.0291	0.2189	~~0.1061~~ 0.330	0.2919	0.00742	~~2.579~~ 2.9244	~~0.008~~ 0.0009
D5 (stream)	8.546	**1.2755**	**17.092**	**7.7690**	**0.7121**	**5.3412**	~~**2.589**~~ **8.062**	**7.1216**	0.1810	~~2.384~~ 2.7033	~~0.0074~~ 0.0008
D6 (ditch)	6.854	**1.0229**	**13.708**	**6.230**	**0.5711**	**4.283**	~~**2.0769**~~ **6.466**	**5.7116**	0.1452	~~18.56~~ 19.1907	~~0.058~~ 0.006
R1 (pond)	~~0.4002~~ 0.3583	~~0.0597~~ 0.0535	~~0.8004~~ 0.7166	~~0.3638~~ 0.3257	~~0.0335~~ 0.0299	~~0.2501~~ 0.2239	~~0.121~~ ~~0.1086~~ 0.338	~~0.3335~~ 0.2986	~~0.0084~~ 0.0076	~~4.35~~ 4.6760	~~0.0135~~ 0.0015
R1 (stream)	~~6.02~~ 4.4650	~~0.8985~~ 0.6664	~~**12.04**~~ **8.93**	~~**5.4727**~~ **4.059**	~~0.5016~~ 0.372	~~**3.76**~~ **2.790**	~~**1.8242**~~ ~~**1.353**~~ **4.212**	~~**5.016**~~ **3.721**	~~0.1275~~ 0.0946	~~11.06~~ 9.9010	~~0.0345~~ 0.0031
R3 (stream)	~~8.359~~ 6.200	~~**1.247**~~ 0.925	~~**16.718**~~ **12.4**	~~**7.5990**~~ **5.6363**	~~**0.6965**~~ 0.5167	~~**5.2243**~~ **3.875**	~~**2.533**~~ ~~**1.879**~~ **5.849**	~~**6.965**~~ **5.167**	~~0.1770~~ 0.1314	~~378.2~~ 358.6000	~~1.181~~ 0.112
R4 (stream)	~~6.057~~ 4.4920	~~0.9040~~ 0.6704	~~**12.114**~~ **8.984**	~~**5.5063**~~ **4.0836**	~~0.5047~~ 0.3743	~~**3.7856**~~ **2.8075**	~~**1.835**~~ ~~**1.3612**~~ **4.238**	~~**5.0475**~~ **3.7433**	~~0.1283~~ 0.0951	~~7.458~~ 6.1200	~~0.0233~~ 0.0019

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold
zRMS: PEC/RAC ratios were re-calculated based on the PEC value accepted in Part B8

For the intended uses, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for fish as characterised by a NOEC for *P. promelas* of 5 µg/L in connection with an assessment factor of 10) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies according to the calculations based on standard Landscape and Mitigation Report.

Table 0-5: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Aclonifen based on FOCUS Step 4 calculations and toxicity data for 1.5L/ha with mitigation of spray drift and run-off for the use of GLOB1310aH in winter cereals at pre-emergence (810g Aclonifen/ha pre-emergence)

Intended use		1.5L formulation/ha pre-emergence								
Active substance		Aclonifen								
Application rate (g/ha)		1 x 810g/ha								
Nozzle reduction	**No-spray buffer (m)**	1/3	5	10	15	20	10	10	10	20
	Vegetated filter strip (m)	-	-	-	-	-	10	50	100	20
None	D1 Ditch	5.1550	1.3960	0.7395	0.5050	0.3839	-	-	-	-
50 %		2.5760	0.6973	0.3694	0.2522	0.1918	-	-	-	-
75 %		1.2870	0.3483	0.1846	0.1260	0.0958	-	-	-	-
90 %		0.5142	0.1392	0.0737	0.0503	0.0383	-	-	-	-
None	D1 stream	6.0760	1.6450	0.8718	0.5954	0.4525	-	-	-	-
50 %		3.0370	0.8219	0.4357	0.2974	0.2261	-	-	-	-
75 %		1.5170	0.4108	0.2176	0.1485	0.1129	-	-	-	-
90 %		0.6061	0.1640	0.0869	0.0593	0.0451	-	-	-	-
None	D2 Ditch	5.1600	1.3970	0.7403	0.5055	0.3843	-	-	-	-
50 %		2.5790	0.6980	0.3698	0.2525	0.1920	-	-	-	-
75 %		1.2880	0.3487	0.1848	0.1261	0.0959	-	-	-	-
90 %		0.5147	0.1393	0.0738	0.0504	0.0383	-	-	-	-
None	D2 Stream	6.1870	1.6750	0.8877	0.6063	0.4608	-	-	-	-
50 %		3.0920	0.8369	0.4437	0.3028	0.2303	-	-	-	-
75 %		1.5450	0.4183	0.2216	0.1513	0.1150	-	-	-	-
90 %		0.6172	0.1670	0.0885	0.0604	0.0459	-	-	-	-
None	D3 Ditch	5.0820	1.3760	0.7290	0.4978	0.3785	-	-	-	-
50 %		2.5400	0.6874	0.3642	0.2487	0.1891	-	-	-	-
75 %		1.2690	0.3434	0.1820	0.1242	0.0944	-	-	-	-
90 %		0.5069	0.1372	0.0727	0.0496	0.0377	-	-	-	-
None	D4 Pond	0.2625	0.1515	0.1089	0.0868	0.0727	-	-	-	-
50 %		0.1311	0.0757	0.0544	0.0433	0.0363	-	-	-	-
75 %		0.0655	0.0378	0.0272	0.0216	0.0181	-	-	-	-
90 %		0.0262	0.0151	0.0108	0.0094	0.0092	-	-	-	-
None	D4 Stream	5.9370	1.6080	0.8519	0.5818	0.4422	-	-	-	-
50 %		2.9670	0.8032	0.4258	0.2906	0.2210	-	-	-	-
75 %		1.4830	0.4014	0.2126	0.1451	0.1104	-	-	-	-

90 %		0.5923	0.1603	0.0849	0.0699	0.0699	-	-	-	-
None	D5 Pond	0.2625	0.1515	0.1089	0.0868	0.0727	-	-	-	-
50 %		0.1311	0.0757	0.0544	0.0433	0.0363	-	-	-	-
75 %		0.0655	0.0378	0.0272	0.0217	0.0181	-	-	-	-
90 %		0.0262	0.0151	0.0109	0.0086	0.0072	-	-	-	-
None	D5 Stream	6.4060	1.7350	0.9192	0.6278	0.4771	-	-	-	-
50 %		3.2020	0.8666	0.4594	0.3135	0.2384	-	-	-	-
75 %		1.6000	0.4331	0.2294	0.1566	0.1191	-	-	-	-
90 %		0.6390	0.1729	0.0916	0.0626	0.0476	-	-	-	-
None	D6 Ditch	5.1380	1.3910	0.7370	0.5033	0.3826	-	-	-	-
50 %		2.5670	0.6949	0.3682	0.2514	0.1963	-	-	-	-
75 %		1.2830	0.3472	0.1963	0.1963	0.1963	-	-	-	-
90 %		0.5125	0.1963	0.1963	0.1963	0.1963	-	-	-	-
None	R1 Pond	0.2928	0.2534	0.2384	0.2307	0.2258	0.1203	0.0941	0.0884	0.0730
50 %		0.2462	0.2269	0.2195	0.2158	0.2134	0.09981	0.0876	0.0848	0.0530
75 %		0.2234	0.2139	0.2103	0.2084	0.2072	0.09041	0.0844	0.0830	0.0468
90 %		0.2099	0.2062	0.2048	0.2040	0.2036	0.08488	0.0825	0.0820	0.0431
None	R1 stream	4.5120	1.2340	1.2340	1.2340	1.2340	0.6474	0.5520	0.5520	0.3361
50 %		2.2550	1.2340	1.2340	1.2340	1.2340	0.552	0.5520	0.5520	0.2873
75 %		1.2340	1.2340	1.2340	1.2340	1.2340	0.552	0.5520	0.5520	0.2873
90 %		1.2340	1.2340	1.2340	1.2340	1.2340	0.552	0.5520	0.5520	0.2873
None	R3 stream	6.2660	1.6970	1.4080	1.4080	1.4080	0.9086	0.6420	0.6420	0.4667
50 %		3.1320	1.4080	1.4080	1.4080	1.4080	0.642	0.6420	0.6420	0.3364
75 %		1.5650	1.4080	1.4080	1.4080	1.4080	0.642	0.6420	0.6420	0.3364
90 %		1.4080	1.4080	1.4080	1.4080	1.4080	0.642	0.6420	0.6420	0.3364
None	R4 stream	4.5400	1.8270	1.8270	1.8270	1.8270	0.9524	0.8238	0.8238	0.4299
50 %		2.2690	1.8270	1.8270	1.8270	1.8270	0.8238	0.8238	0.8238	0.4299
75 %		1.8270	1.8270	1.8270	1.8270	1.8270	0.8238	0.8238	0.8238	0.4299
90 %		1.8270	1.8270	1.8270	1.8270	1.8270	0.8238	0.8238	0.8238	0.4299
RAC (µg/L)										
0.5 (fish growth)		**PEC/RAC ratio**								
None	D1 Ditch	**10.3100**	**2.7920**	**1.4790**	**1.0100**	0.7678	-	-	-	-
50 %		**5.1520**	**1.3946**	0.7388	0.5044	0.3836	-	-	-	-
75 %		**2.5740**	0.6966	0.3692	0.2520	0.1916	-	-	-	-
90 %		**1.0284**	0.2784	0.1474	0.1007	0.0765	-	-	-	-
None	D1 stream	**12.1520**	**3.2900**	**1.7436**	**1.1908**	0.9050	-	-	-	-
50 %		**6.0740**	**1.6438**	0.8714	0.5948	0.4522	-	-	-	-
75 %		**3.0340**	0.8216	0.4352	0.2970	0.2258	-	-	-	-
90 %		**1.2122**	0.3280	0.1738	0.1187	0.0902	-	-	-	-
None	D2 Ditch	**10.3200**	**2.7940**	**1.4806**	**1.0110**	0.7686	-	-	-	-
50 %		**5.1580**	**1.3960**	0.7396	0.5050	0.3840	-	-	-	-

75 %		**2.5760**	0.6974	0.3696	0.2522	0.1918	-	-	-	-
90 %		**1.0294**	0.2786	0.1476	0.1008	0.0767	-	-	-	-
None	D2 Stream	**12.3740**	**3.3500**	**1.7754**	**1.2126**	0.9216	-	-	-	-
50 %		**6.1840**	**1.6738**	0.8874	0.6056	0.4606	-	-	-	-
75 %		**3.0900**	0.8366	0.4432	0.3026	0.2300	-	-	-	-
90 %		**1.2344**	0.3340	0.1770	0.1209	0.0919	-	-	-	-
None	D3 Ditch	**10.1640**	**2.7520**	**1.4580**	0.9956	0.7570	-	-	-	-
50 %		**5.0800**	**1.3748**	0.7284	0.4974	0.3782	-	-	-	-
75 %		**2.5380**	0.6868	0.3640	0.2484	0.1889	-	-	-	-
90 %		**1.0138**	0.2744	0.1453	0.0992	0.0754	-	-	-	-
None	D4 Pond	0.5250	0.3030	0.2178	0.1735	0.1453	-	-	-	-
50 %		0.2622	0.1514	0.1087	0.0866	0.0726	-	-	-	-
75 %		0.1310	0.0756	0.0543	0.0433	0.0362	-	-	-	-
90 %		0.0523	0.0302	0.0217	0.0189	0.0184	-	-	-	-
None	D4 Stream	**11.8740**	**3.2160**	**1.7038**	**1.1636**	0.8844	-	-	-	-
50 %		**5.9340**	**1.6064**	0.8516	0.5812	0.4420	-	-	-	-
75 %		**2.9660**	0.8028	0.4252	0.2902	0.2208	-	-	-	-
90 %		**1.1846**	0.3206	0.1698	0.1397	0.1397	-	-	-	-
None	D5 Pond	0.5250	0.3030	0.2178	0.1735	0.1453	-	-	-	-
50 %		0.2622	0.1514	0.1088	0.0867	0.0726	-	-	-	-
75 %		0.1310	0.0756	0.0543	0.0433	0.0363	-	-	-	-
90 %		0.0523	0.0302	0.0217	0.0173	0.0145	-	-	-	-
None	D5 Stream	**12.8120**	**3.4700**	**1.8384**	**1.2556**	0.9542	-	-	-	-
50 %		**6.4040**	**1.7332**	0.9188	0.6270	0.4768	-	-	-	-
75 %		**3.2000**	0.8662	0.4588	0.3132	0.2382	-	-	-	-
90 %		**1.2780**	0.3458	0.1833	0.1251	0.0951	-	-	-	-
None	D6 Ditch	**10.2760**	**2.7820**	**1.4740**	**1.0066**	0.7652	-	-	-	-
50 %		**5.1340**	**1.3898**	0.7364	0.5028	0.3926	-	-	-	-
75 %		**2.5660**	0.6944	0.3926	0.3926	0.3926	-	-	-	-
90 %		**1.0250**	0.3926	0.3926	0.3926	0.3926	-	-	-	-
None	R1 Pond	0.5856	0.5068	0.4768	0.4614	0.4516	0.2406	0.1881	0.1769	0.1460
50 %		0.4924	0.4538	0.4390	0.4316	0.4268	0.1996	0.1752	0.1696	0.1060
75 %		0.4468	0.4278	0.4206	0.4168	0.4144	0.1808	0.1688	0.1661	0.0935
90 %		0.4198	0.4124	0.4096	0.4080	0.4072	0.1698	0.1650	0.1640	0.0862
None	R1 stream	**9.0240**	**2.4680**	**2.4680**	**2.4680**	**2.4680**	**1.2948**	**1.1040**	**1.1040**	0.6722
50 %		**4.5100**	**2.4680**	**2.4680**	**2.4680**	**2.4680**	**1.1040**	**1.1040**	**1.1040**	0.5746
75 %		**2.4680**	**2.4680**	**2.4680**	**2.4680**	**2.4680**	**1.1040**	**1.1040**	**1.1040**	0.5746
90 %		**2.4680**	**2.4680**	**2.4680**	**2.4680**	**2.4680**	**1.1040**	**1.1040**	**1.1040**	0.5746
None	R3 stream	**12.5320**	**3.3940**	**2.8160**	**2.8160**	**2.8160**	**1.8172**	**1.2840**	**1.2840**	0.9334
50 %		**6.2640**	**2.8160**	**2.8160**	**2.8160**	**2.8160**	**1.2840**	**1.2840**	**1.2840**	0.6728
75 %		**3.1300**	**2.8160**	**2.8160**	**2.8160**	**2.8160**	**1.2840**	**1.2840**	**1.2840**	0.6728

90 %		**2.8160**	**2.8160**	**2.8160**	**2.8160**	**2.8160**	**1.2840**	**1.2840**	**1.2840**	0.6728
None	R4 stream	**9.0800**	**3.6540**	**3.6540**	**3.6540**	**3.6540**	**1.9048**	**1.6476**	**1.6476**	0.8598
50 %		**4.5380**	**3.6540**	**3.6540**	**3.6540**	**3.6540**	**1.6476**	**1.6476**	**1.6476**	0.8598
75 %		**3.6540**	**3.6540**	**3.6540**	**3.6540**	**3.6540**	**1.6476**	**1.6476**	**1.6476**	0.8598
90 %		**3.6540**	**3.6540**	**3.6540**	**3.6540**	**3.6540**	**1.6476**	**1.6476**	**1.6476**	0.8598

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 0-6: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Aclonifen based on FOCUS Step 4 calculations and toxicity data for 2L/ha with mitigation of spray drift and run-off for the use of GLOB1310aH in winter cereals at pre-emergence (1080g Aclonifen/ha pre-emergence)

Intended use		2L formulation/ha pre-emergence										
Active substance		Aclonifen										
Application rate (g/ha)		1 x 1080g/ha										
Nozzle reduction	**No-spray buffer (m)**	1/3	5	10	15	20	50	10	10	10	20	20
	Vegetated filter strip (m)	-	-	-	-	-	-	10	50	100	20	50
None	D1 Ditch	6.8780	1.8610	0.9864	0.6735	0.5122	0.2118	-	-	-	-	-
50 %		3.4360	0.9299	0.4928	0.3365	0.2558	-	-	-	-	-	-
75 %		1.7170	0.4647	0.2462	0.1681	0.1278	-	-	-	-	-	-
90 %		0.6861	0.1856	0.0983	0.0671	0.0511	-	-	-	-	-	-
None	D1 stream	8.1060	2.1940	1.1630	0.7940	0.6038	0.2497	-	-	-	-	-
50 %		4.0500	1.0960	0.5808	0.3965	0.3016	-	-	-	-	-	-
75 %		2.0240	0.5478	0.2902	0.1981	0.1506	-	-	-	-	-	-
90 %		0.8086	0.2188	0.1159	0.0792	0.0602	-	-	-	-	-	-
None	D2 Ditch	6.8840	1.8630	0.9874	0.6743	0.5128	0.2121	-	-	-	-	-
50 %		3.4400	0.9309	0.4933	0.3369	0.2562	-	-	-	-	-	-
75 %		1.7180	0.4653	0.2465	0.1683	0.1280	-	-	-	-	-	-
90 %		0.6868	0.1859	0.0985	0.0673	0.0512	-	-	-	-	-	-
None	D2 Stream	8.2540	2.2340	1.1840	0.8085	0.6149	0.2543	-	-	-	-	-
50 %		4.1240	1.1160	0.5914	0.4038	0.3071	-	-	-	-	-	-
75 %		2.0610	0.5578	0.2955	0.2018	0.1534	-	-	-	-	-	-
90 %		0.8234	0.2228	0.1181	0.0806	0.0613	-	-	-	-	-	-
None	D3 Ditch	6.7800	1.8350	0.9724	0.6640	0.5050	0.2088	-	-	-	-	-
50 %		3.3880	0.9167	0.4858	0.3317	0.2522	-	-	-	-	-	-
75 %		1.6920	0.4581	0.2427	0.1657	0.1260	-	-	-	-	-	-
90 %		0.6763	0.1830	0.0969	0.0662	0.0503	-	-	-	-	-	-
None	D4 Pond	0.3502	0.2021	0.1452	0.1157	0.0969	0.0504	-	-	-	-	-
50 %		0.1749	0.1010	0.0725	0.0578	0.0484	-	-	-	-	-	-

75 %		0.0874	0.0504	0.0362	0.0289	0.0242	-	-	-	-	-	-
90 %		0.0349	0.0201	0.0145	0.0137	0.0133	-	-	-	-	-	-
None	D4 Stream	7.9210	2.1440	1.1360	0.7759	0.5900	0.2440	-	-	-	-	-
50 %		3.9580	1.0710	0.5676	0.3875	0.2947	-	-	-	-	-	-
75 %		1.9770	0.5353	0.2836	0.1936	0.1472	-	-	-	-	-	-
90 %		0.7902	0.2138	0.1133	0.1009	0.1009	-	-	-	-	-	-
None	D5 Pond	0.3503	0.2021	0.1453	0.1157	0.0969	0.0504	-	-	-	-	-
50 %		0.1749	0.1010	0.0726	0.0578	0.0484	-	-	-	-	-	-
75 %		0.0874	0.0504	0.0362	0.0289	0.0242	-	-	-	-	-	-
90 %		0.0349	0.0201	0.0145	0.0115	0.0097	-	-	-	-	-	-
None	D5 Stream	8.5460	2.3140	1.2260	0.8372	0.6366	0.2633	-	-	-	-	-
50 %		4.2700	1.1560	0.6124	0.4181	0.3180	-	-	-	-	-	-
75 %		2.1340	0.5775	0.3060	0.2089	0.1588	-	-	-	-	-	-
90 %		0.8526	0.2307	0.1222	0.0835	0.0635	-	-	-	-	-	-
None	D6 Ditch	6.8540	1.8550	0.9830	0.6712	0.5105	0.2992	-	-	-	-	-
50 %		3.4250	0.9268	0.4911	0.3353	0.2992	-	-	-	-	-	-
75 %		1.7110	0.4632	0.2992	0.2992	0.2992	-	-	-	-	-	-
90 %		0.6837	0.2992	0.2992	0.2992	0.2992	-	-	-	-	-	-
None	R1 Pond	0.4002	0.3471	0.3270	0.3166	0.3100	0.2939	0.1167	0.1290	0.1214	0.0974	0.0733
50 %		0.3374	0.3114	0.3015	0.2964	0.2932	-	0.8636	0.1203	0.1166	0.0726	0.0645
66%		-	-	-	-	-	-	0.7625	-	-	-	-
75 %		0.3067	0.2939	0.2890	0.2865	0.2849	-	0.7625	0.1160	0.1142	0.0642	0.0602
90 %		0.2886	0.2835	0.2816	0.2806	0.2800	-	0.7625	0.1135	0.1128	0.0593	0.0577
None	R1 stream	6.0200	1.7050	1.7050	1.7050	1.7050	1.7050	0.7625	0.7625	0.7625	0.4484	0.3968
50 %		3.0080	1.7050	1.7050	1.7050	1.7050	-	1.199	0.7625	0.7625	0.3968	0.3968
66%		-	-	-	-	-	-	0.8895	-	-	-	-
75 %		1.7050	1.7050	1.7050	1.7050	1.7050	-	0.8895	0.7625	0.7625	0.3968	0.3968
90 %		1.7050	1.7050	1.7050	1.7050	1.7050	-	0.8895	0.7625	0.7625	0.3968	0.3968
None	R3 stream	8.3590	2.2630	1.9510	1.9510	1.9510	1.9510	0.8895	0.8895	0.8895	0.6227	0.4663
50 %		4.1770	1.9510	1.9510	1.9510	1.9510	-	1.138	0.8895	0.8895	0.4663	0.4663
66%		-	-	-	-	-	-	1.138	-	-	-	-
75 %		2.0870	1.9510	1.9510	1.9510	1.9510	-	1.138	0.8895	0.8895	0.4663	0.4663
90 %		1.9510	1.9510	1.9510	1.9510	1.9510	-	1.138	0.8895	0.8895	0.4663	0.4663
None	R4 stream	6.0570	2.5230	2.5230	2.5230	2.5230	2.5230	1.138	1.1380	1.1380	0.5939	0.5939
50 %		3.0260	2.5230	2.5230	2.5230	2.5230	-	-	1.1380	1.1380	0.5939	0.5939
66%		-	-	-	-	-	-	-	-	-	-	-
75 %		2.5230	2.5230	2.5230	2.5230	2.5230	-	-	1.1380	1.1380	0.5939	0.5939
90 %		2.5230	2.5230	2.5230	2.5230	2.5230	-	-	1.1380	1.1380	0.5939	0.5939
RAC (µg/L)												

0.5 (fish growth)		**PEC/RAC ratio**										
None	D1 Ditch	**13.7560**	**3.7220**	**1.9728**	**1.3470**	**1.0244**	0.4236	-	-	-	-	-
50 %		**6.8720**	**1.8598**	0.9856	0.6730	0.5116	-	-	-	-	-	-
75 %		**3.4340**	0.9294	0.4924	0.3362	0.2556	-	-	-	-	-	-
90 %		**1.3722**	0.3712	0.1967	0.1343	0.1021	-	-	-	-	-	-
None	D1 stream	**16.2120**	**4.3880**	**2.3260**	**1.5880**	**1.2076**	0.4994	-	-	-	-	-
50 %		**8.1000**	**2.1920**	**1.1616**	0.7930	0.6032	-	-	-	-	-	-
75 %		**4.0480**	**1.0956**	0.5804	0.3962	0.3012	-	-	-	-	-	-
90 %		**1.6172**	0.4376	0.2318	0.1583	0.1204	-	-	-	-	-	-
None	D2 Ditch	**13.7680**	**3.7260**	**1.9748**	**1.3486**	**1.0256**	0.4242	-	-	-	-	-
50 %		**6.8800**	**1.8618**	0.9866	0.6738	0.5124	-	-	-	-	-	-
75 %		**3.4360**	0.9306	0.4930	0.3366	0.2560	-	-	-	-	-	-
90 %		**1.3736**	0.3718	0.1970	0.1346	0.1024	-	-	-	-	-	-
None	D2 Stream	**16.5080**	**4.4680**	**2.3680**	**1.6170**	**1.2298**	0.5086	-	-	-	-	-
50 %		**8.2480**	**2.2320**	**1.1828**	0.8076	0.6142	-	-	-	-	-	-
75 %		**4.1220**	**1.1156**	0.5910	0.4036	0.3068	-	-	-	-	-	-
90 %		**1.6468**	0.4456	0.2362	0.1612	0.1226	-	-	-	-	-	-
None	D3 Ditch	**13.5600**	**3.6700**	**1.9448**	**1.3280**	**1.0100**	0.4176	-	-	-	-	-
50 %		**6.7760**	**1.8334**	0.9716	0.6634	0.5044	-	-	-	-	-	-
75 %		**3.3840**	0.9162	0.4854	0.3314	0.2520	-	-	-	-	-	-
90 %		**1.3526**	0.3660	0.1939	0.1324	0.1007	-	-	-	-	-	-
None	D4 Pond	0.7004	0.4042	0.2904	0.2314	0.1938	0.1009	-	-	-	-	-
50 %		0.3498	0.2020	0.1451	0.1156	0.0968	-	-	-	-	-	-
75 %		0.1747	0.1008	0.0725	0.0577	0.0483	-	-	-	-	-	-
90 %		0.0698	0.0403	0.0289	0.0274	0.0267	-	-	-	-	-	-
None	D4 Stream	**15.8420**	**4.2880**	**2.2720**	**1.5518**	**1.1800**	0.4880	-	-	-	-	-
50 %		**7.9160**	**2.1420**	**1.1352**	0.7750	0.5894	-	-	-	-	-	-
75 %		**3.9540**	**1.0706**	0.5672	0.3872	0.2944	-	-	-	-	-	-
90 %		**1.5804**	0.4276	0.2266	0.2018	0.2018	-	-	-	-	-	-
None	D5 Pond	0.7006	0.4042	0.2906	0.2314	0.1939	0.1009	-	-	-	-	-
50 %		0.3498	0.2020	0.1451	0.1156	0.0968	-	-	-	-	-	-
75 %		0.1748	0.1009	0.0725	0.0577	0.0484	-	-	-	-	-	-
90 %		0.0698	0.0403	0.0289	0.0231	0.0193	-	-	-	-	-	-
None	D5 Stream	**17.0920**	**4.6280**	**2.4520**	**1.6744**	**1.2732**	0.5266	-	-	-	-	-
50 %		**8.5400**	**2.3120**	**1.2248**	0.8362	0.6360	-	-	-	-	-	-
75 %		**4.2680**	**1.1550**	0.6120	0.4178	0.3176	-	-	-	-	-	-
90 %		**1.7052**	0.4614	0.2444	0.1669	0.1269	-	-	-	-	-	-
None	D6 Ditch	**13.7080**	**3.7100**	**1.9660**	**1.3424**	**1.0210**	0.5984	-	-	-	-	-
50 %		**6.8500**	**1.8536**	0.9822	0.6706	0.5984	-	-	-	-	-	-

75 %		**3.4220**	0.9264	0.5984	0.5984	0.5984	-	-	-	-	-	-
90 %		**1.3674**	0.5984	0.5984	0.5984	0.5984	-	-	-	-	-	-
None	R1 Pond	0.8004	0.6942	0.6540	0.6332	0.6200	0.5878	0.3252	0.2580	0.2428	0.1947	0.1465
50 %		0.6748	0.6228	0.6030	0.5928	0.5864	-	0.2736	0.2406	0.2332	0.1451	0.1290
75 %		0.6134	0.5878	0.5780	0.5730	0.5698	-	0.2482	0.2320	0.2284	0.1283	0.1205
90 %		0.5772	0.5670	0.5632	0.5612	0.5600	-	0.2334	0.2270	0.2256	0.1185	0.1154
None	R1 stream	**12.0400**	**3.4100**	**3.4100**	**3.4100**	**3.4100**	**3.4100**	**1.7272**	**1.5250**	**1.5250**	0.8968	0.7936
50 %		**6.0160**	**3.4100**	**3.4100**	**3.4100**	**3.4100**	-	**1.5250**	**1.5250**	**1.5250**	0.7936	0.7936
75 %		**3.4100**	**3.4100**	**3.4100**	**3.4100**	**3.4100**	-	**1.5250**	**1.5250**	**1.5250**	0.7936	0.7936
90 %		**3.4100**	**3.4100**	**3.4100**	**3.4100**	**3.4100**	-	**1.5250**	**1.5250**	**1.5250**	0.7936	0.7936
None	R3 stream	**16.7180**	**4.5260**	**3.9020**	**3.9020**	**3.9020**	**3.9020**	**2.3980**	**1.7790**	**1.7790**	**1.2454**	0.9326
50 %		**8.3540**	**3.9020**	**3.9020**	**3.9020**	**3.9020**	-	**1.7790**	**1.7790**	**1.7790**	0.9326	0.9326
75 %		**4.1740**	**3.9020**	**3.9020**	**3.9020**	**3.9020**	-	**1.7790**	**1.7790**	**1.7790**	0.9326	0.9326
90 %		**3.9020**	**3.9020**	**3.9020**	**3.9020**	**3.9020**	-	**1.7790**	**1.7790**	**1.7790**	0.9326	0.9326
None	R4 stream	**12.1140**	**5.0460**	**5.0460**	**5.0460**	**5.0460**	**5.0460**	**2.2760**	**2.2760**	**2.2760**	**1.1878**	**1.1878**
50 %		**6.0520**	**5.0460**	**5.0460**	**5.0460**	**5.0460**	-	**2.2760**	**2.2760**	**2.2760**	**1.1878**	**1.1878**
75 %		**5.0460**	**5.0460**	**5.0460**	**5.0460**	**5.0460**	-	**2.2760**	**2.2760**	**2.2760**	**1.1878**	**1.1878**
90 %		**5.0460**	**5.0460**	**5.0460**	**5.0460**	**5.0460**	-	**2.2760**	**2.2760**	**2.2760**	**1.1878**	**1.1878**

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

zRMS comments:
Aclonifen
For the application rate of 1.5L formulation/ha (810 g aclonifen/ha), based on the PEC FOCUS Step 4 values the risk is accepted for all scenarios when the appropriate risk mitigation measures have been applied.

For the application rate of 2L formulation/ha (1080 g aclonifen/ha), based on the PEC FOCUS Step 4 values the risk is accepted for scenarios D1 Ditch, D1 Stream, D2 Ditch, D2 Stream, D3 Ditch, D4 Pond, D4 Stream, D5 Pond, D5 Stream, D6 Ditch, R1 Pond, R1 Stream, R3 Stream when the appropriate risk mitigation measures have been applied. In the case of R4 Stream, the risk is unacceptable.

PEC/RAC calculations with Step 4 VFSMOD

In addition to the calculations presented above based on the standard FOCUS Landscape and Mitigation Report Step 4, the applicant also used VFSMod calculations to reduce run-off as this tool is now accepted in some Member States. PEC/RAC calculations with VFSMod are presented below as a Tier 2 refinement.

Table 0-7: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aclonifen for each organism group based on FOCUS Step 4 VFSMOD calculations for the use of 1.5L GLOB1310aH/ha in winter cereals at pre-emergence (810g Aclonifen/ha)

PECsw (µg/L)	**Scenario**	**STEP 4 Aclonifen**	
Nozzle reduction	Vegetative strip (m)	10	20

	No spray buffer (m)	10	20
None	R1 pond	0.1088	0.0726
50 %		0.0543	0.0363
75 %		0.0271	0.0181
90 %		0.0108	0.0072
None	R1 stream	0.6474	0.3360
50 %		0.3235	0.1679
75 %		0.1616	0.0839
90 %		0.0645	0.0335
None	R3 stream	0.8991	0.4667
50 %		0.5188	0.3364
75 %		0.5188	0.3364
90 %		0.5188	0.3364
None	R4 stream	0.6514	0.3381
50 %		0.3255	0.1689
75 %		0.1626	0.0844
90 %		0.0649	0.0337
RAC (µg/L)			
0.5 (fish growth)		**PEC/RAC ratio**	
None	R1 pond	0.2176	0.1452
50 %		0.1087	0.0725
75 %		0.0543	0.0362
90 %		0.0217	0.0145
None	R1 stream	**1.2948**	0.6720
50 %		0.6470	0.3358
75 %		0.3232	0.1677
90 %		0.1291	0.0670
None	R3 stream	**1.7982**	0.9334
50 %		**1.0376**	0.6728
75 %		**1.0376**	0.6728
90 %		**1.0376**	0.6728
None	R4 stream	**1.3028**	0.6762
50 %		0.6510	0.3378
75 %		0.3252	0.1687
90 %		0.1299	0.0674

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 0-8: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for aclonifen for each organism group based on FOCUS Step 4 VFSMOD calculations for the use of 2L GLOB1310aH/ha in winter cereals at pre-emergence (1080g Aclonifen/ha)

PECsw (µg/L)	**Scenario**	**STEP 4 Aclonifen**	
Nozzle reduction	Vegetative strip (m)	10	20
	No spray buffer (m)	10	20
None	R1 pond	0.1451	0.0968
50 %		0.0725	0.0484
75 %		0.0362	0.0242
90 %		0.0147	0.0096
None	R1 stream	0.8636	0.4484
50 %		0.4313	0.2240
75 %		0.2155	0.1119
90 %		0.0861	0.0447
None	R3 stream	1.1990	0.6227
50 %		0.7184	0.4659
75 %		0.7184	0.4659
90 %		0.7184	0.4659
None	R4 stream	0.8689	0.4511
50 %		0.4340	0.2253
75 %		0.2168	0.1125
90 %		0.0866	0.0450
RAC (µg/L)			
0.5 (fish growth)		**PEC/RAC ratio**	
None	R1 pond	0.2902	0.1937
50 %		0.1450	0.0967
75 %		0.0724	0.0483
90 %		0.0293	0.0193
None	R1 stream	**1.7272**	0.8968
50 %		0.8626	0.4480
75 %		0.4310	0.2238
90 %		0.1722	0.0894
None	R3 stream	**2.3980**	**1.2454**
50 %		**1.4368**	0.9318
75 %		**1.4368**	0.9318
90 %		**1.4368**	0.9318
None	R4 stream	**1.7378**	0.9022
50 %		0.8680	0.4506
75 %		0.4336	0.2250
90 %		0.1733	0.0899

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

zRMS comments: The use of VFSMod should be considered at the Member State level.
When this approach is accepted the risk is acceptable for the following scenarios:

- for the application rate of 1.5L formulation/ha (810 g aclonifen/ha)
  - R1 pound with 10 m vegetative strip,
  - R1 stream with 10 m vegetative strip and 50% drift reduction or 20 m vegetative strip,
  - R3 stream with 20 m vegetative strip,
  - R4 stream with 10 m vegetative strip and 50% drift reduction or 20 m vegetative strip.

- for the application rate of 2L formulation/ha (1080 g aclonifen/ha)
  - R1 pound with 10 m vegetative strip,
  - R1 stream with 10 m vegetative strip and 50% drift reduction or 20 m vegetative strip,
  - R3 stream with 20 m vegetative strip and 50% drift reduction,
  - R4 stream with 10 m vegetative strip and 50% drift reduction or 20 m vegetative strip.

Exposure pattern analysis (EPAT)

The RAC of 0.5 µg/L is derived from an early life-stage fish study that lasted 35 days, with a standard assessment factor of 10. The EU agreed NOEC of 5 µg/L is based on growth, whereas other parameters like hatch and fry survival were not affected at this concentration. A NOEChatch of 11 µg/L was indeed found in this ELS study, which would have resulted in a RAC of 1.1 µg/L. In addition, the ELS study must be considered as very conservative study because it is a flow-through system wherein the active ingredient is renewed every 3-4 days, and the duration of the study is 35 days. The exposure of fish in this study is therefore very conservative; it led to an overestimation of the risk to fish, and to an unrealistic endpoint (5µg/L). In the environment, the fish would never be exposed to a constant concentration of active ingredient for a duration of 35 days. The overall survival is not affected and growth effects of aclonifen are known to be the consequence of feeding disorder.

To demonstrate what would be a more realistic exposure of fish to aclonifen derived from the use of GLOB1310aH, the applicant used EPAT v1.2 (Exposure Pattern Analysis Tool). EPAT v.1.2 analysed the duration of the exposure event (an "event" occurring whenever the concentration exceeds the RAC). Thus, an event is defined as a concentration $\geq$0.5 µg Aclonifen/L. Based on its outcome, the applicant compares the realistic exposure in field, to the exposure from the chronic fish study on which the end-point of 5 µg/L is based.

The EPAT results presented in the tables below were obtained from the analysis of the results arising from the FOCUS Step 4 PECsw calculations according to the Landscape and Mitigation Report. The PECsw values obtained with the VFSMod are not considered in these EPAT analysis.

For the use 1 (1.5L GLOB1310aH/ha, equivalent to 810g Aclonifen/ha at pre-emergence), the PECsw in all scenarios is below the lowest RAC of aclonifen, namely 0.5 µg a.s./ha, when 20m vegetated buffer strip that is also a 20m no spray buffer is respected. When a 10m vegetated buffer strip that is also a 10m no spray buffer in combination with at least 50% drift reduction technology, the PECsw is below the RAC in all scenarios, except R1 Stream, R3 stream, and R4 Stream. Similarly, when a 10m vegetated buffer strip in combination with a 50m no spray buffer, the PECsw is below the RAC in all scenarios, except R1 Stream, R3 stream, and R4 Stream. Thus, the EPAT analysis was done for these 3 scenarios to show that the exposure to fish to aclonifen is acceptable.

Table 9.5-9: Event summary for Step 4 scenarios R1 stream, R3 stream and R4 stream for use 1 of GLOB1310aH (Aclonifen, 810 g/ha at pre-emergence)

Mitigation	Scenario	Number of	Vent num-	Max. conc.	Duration	Interval	Total du-

measured applied.		events	ber	(µg L-1)	of event (d)	between events (d)	ration of events (d)
10m vbs 10m nsb 50%drt	R1 Stream	2	1	0.5520	0.292	-	0.885
			2	0.5201	0.583	35.667	
	R3 Stream	6	1	0.642	0.458	-	2.167
			2	0.6147	0.875	0.542	
			3	0.5275	0.125	0.166	
			4	0.5875	0.333	0.834	
			5	0.5185	0.334	16.708	
			6	0.5002	0.042	9.666	
	R4 Stream	3	1	0.8238	0.541	-	1.583
			2	0.765	0.417	0.5	
			3	0.5432	0.625	87.542	

Vbs: vegetated buffer strip; nsb: no spray buffer; drt: drift reduction technology

From the table above, the risk for aquatic organisms for Use 1 (1.5L formulation/ha, equivalent to 810 g Aclonifen/ha pre-emergence) with 10m vegetated buffer strip + 10m no spray buffer + 50% drift reduction nozzles is acceptable because:

- Only 2, 6 and 3 peaks in R1, R3 and R4 respectively of short duration above the RAC of 0.5 µg/L.
- Exceedance of RAC occurs during 0.885, 2.167, 1.583 days in R1, R3 and R4 respectively. A maximum exposure of 2.167 d would not be long enough to produce the effects observed in the early lifestage toxicity study (35 days, flow trough) conducted with *Pimephales promelas*.

For the use 2 (2.0L GLOB1310aH/ha, equivalent to 1080g Aclonifen/ha + 120 g Flufenacet/ha at pre-emergence), when a 10m vegetated buffer strip that is also a 10m no spray buffer in combination with at least 75% drift reduction technology is applied the PECsw in all scenarios (except R1 stream, R3 stream, R4 stream) is below the lowest RAC of aclonifen, namely 0.5 µg a.s./ha. When a 20m vegetated buffer strip that is also a 20m no spray buffer in combination with at least 50% drift reduction technology, the PECsw is below the RAC in all scenarios, except R4 Stream. Thus, the EPAT analysis was done for these three mitigation measures.

Table 9.5-10: Event summary for Step 4 scenarios R1 stream, R3 stream and R4 stream for use ~~3~~ 2 of GLOB1310aH (Aclonifen, 1080 g/ha at pre-emergence)

Mitigation measured applied.	Scenario	Number of events	Vent number	Max. conc. (µg L-1)	Duration of event (d)	Interval between events (d)	Total duration of events (d)
10m vbs 10m nsb 75%drt	R1 Stream	6	1	0.7625	0.375	-	2.168
			2	0.6136	0.334	33.625	
			3	0.6185	0.334	0.666	
			4	0.7151	0.625	0.625	
			5	0.6796	0.291	32.375	
			6	0.5126	0.209	7.75	
	R3 Stream	6	1	0.8895	0.458	-	2.834
			2	0.851	0.916	0.542	
			3	0.7302	0.209	0.125	
			4	0.8132	0.333	0.75	
			5	0.7162	0.459	16.708	
			6	0.6902	0.459	9.541	
	R4 Stream	6	1	1.138	0.541	-	3.292
			2	1.055	0.417	0.5	
			3	0.7451	0.708	87.542	
			4	0.5469	0.292	2.375	
			5	0.6041	1	23.625	
			6	0.532	0.334	0.041	
20m vbs 20m nsb 50%drt	R4 Stream	2	1	0.5939	0.417	-	0.751
			2	0.549	0.334	0.583	
			2	0.549	0.334	0.583	

Vbs: vegetated buffer strip; nsb: no spray buffer; drt: drift reduction technology

From the table above, the risk for aquatic organisms for Use 2 (2.0L formulation/ha, equivalent to 1080 g Aclonifen/ha at pre-emergence) with 10m vegetated buffer strip + 10m no spray buffer + 66% drift reduction nozzles or 75% drift reduction nozzles is acceptable because:

- Only 6 peaks in R1, R3 and R4 of short duration above the RAC of 0.5 µg/L.
- Exceedance of RAC occurs during 2.168, 2.834, 3.292 days in R1, R3 and R4 respectively. A maximum exposure of 3.292 h would not be long enough to produce the effects observed in the early lifestage toxicity study (35 days, flow trough) conducted with *Pimephales promelas*.

The risk for aquatic organisms for Use ~~3~~ 2 (2.0L formulation/ha, equivalent to 1080 g Aclonifen/ha at pre-emergence) with 20m vegetated buffer strip + 20m no spray buffer is acceptable because + 50% drift reduction nozzles:

- Only 2 peaks in R4 of short duration above the RAC of 0.5 µg/L and the other minor peaks (<0.5µg/L) occur several months later
- Exceedance of RAC occurs during 0.751 days. A maximum exposure of 0.751 d would not be long enough to produce the effects observed in the early lifestage toxicity study (35 days, flow trough) conducted with *Pimephales promelas*.

Exposure pattern analysis (EPAT) of PECsw Step 4 obtained with VFSMod simulations

An additional refinement approach is presented by the applicant. This consists in the exposure pattern analysis of the PECsw a in those scenarios in which the maximum RAC of 0.5 µg Aclonifen/L is exceeded. The results are summarized in the tables below.

Table 9.5-11: Event summary for Step 4-VFSMod in scenarios R1 stream, R3 stream and R4 stream for use 3 of GLOB1310aH (Aclonifen, 1080 g/ha at pre-emergence)

Mitigation measured applied.	Scenario	Number of events	Vent number	Max. conc. (µg L-1)	Duration of event (d)	Interval between events (d)	Total duration of events (d)
10m vbs 10m nsb	R1 Stream	1	1	0.8636	0.167	-	0.167
	R3 Stream	2	1	1.199	0.208	-	1.124
			2	0.7184	0.916	9.459	
	R4 Stream	1	1	0.8689	0.208	-	0.208
20m vbs 20m nsb	R3 Stream	1	1	0.6227	0.125	-	0.125

Vbs: vegetated buffer strip; nsb: no spray buffer; drt: drift reduction technology

From the table above, the risk for aquatic organisms for Use ~~3~~ 2 (2.0L formulation/ha, equivalent to 1080 g Aclonifen/ha at pre-emergence) with 10m vegetated buffer strip + 10m no spray buffer is acceptable because:

- Only 1, 2 and 2 peaks in R1, R3 and R4 respectively of short duration above the RAC of 0.5 µg/L.
- Exceedance of RAC occurs during 0.167, 1.124, 0.208 days in R1, R3 and R4 respectively. A maximum exposure of 1.124 d would not be long enough to produce the effects observed in the early lifestage toxicity study (35 days, flow trough) conducted with *Pimephales promelas*.

The risk for aquatic organisms for Use ~~3~~ 2 (2.0L formulation/ha, equivalent to 1080 g Aclonifen/ha at pre-emergence) with 20m vegetated buffer strip + 20m no spray buffer is acceptable:

- Only 1 peaks in R3 of short duration above the RAC of 0.5 µg/L and the other minor peaks (<0.5µg/L) occur several months later
- Exceedance of RAC occurs during 0.125 days. A maximum exposure of 0.125 d would not be long enough to produce the effects observed in the early lifestage toxicity study (35 days, flow trough) conducted with *Pimephales promelas*.

zRMS comments: In order to refine the chronic risk to aquatic organisms the Applicant conducted the an Exposure Pattern Analysis (EPAT). EPAT is an evaluation tool specifically designed for the analysis of TOXSWA output files from the surface water model FOCUS TOXSWA, what is currently not analysed further. The EPAT analysed how often a given concentration is reached or how long a concentration may be expected to remain above a given threshold.
https://rifcon.de/wp-content/uploads/2018/08/rifcon_epat_2010.pdf

The Exposure Pattern Analysis has been assessed in Part B8 and can be considered as supporting information. This should be considered at the Member State level.

9.5.2.2 Flufenacet

Table 0-12: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for flufenacet (90g flufenacet/ha) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of 1.5L/ha of GLOB1310aH in winter cereals at pre-emergence

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic macro-phytes**	**Higher Tier**
Test species		*L. macrochirus*	*O. mykiss*	*D. magna*	*D. magna*	*P. subcapitata*	*L gibba*	*Microcosms*
Endpoint (µg/L)		LC_{50}	NOEC	LC_{50}	NOEC	EC_{50}	ErC_{50}	NOEC
		2130	200	30900	3260	4.5	2.43	12
AF		100	10	100	10	10	10	5
RAC (µg/L)		21.3	20	309	326	0.45	0.243	2.4
FOCUS Scenario	**PEC max (µg/L)**							
Step 1								
Worst-case	24.8405	**1.1662**	**1.242**	0.080389968	0.076197853	**55.201**	**102.22**	0.2915
Step 2								
N-Europe	10.8204	0.508	0.54102	0.035017476	0.033191411	**24.045**	**44.5283**	0.127
S-Europe	8.7905	0.412699531	0.439525	0.02844822	0.026964724	**19.53**	**36.17489**	0.1031
Step 3								
D1 (ditch)	2.863	0.1344	0.1432	0.0093	0.0088	**6.3622**	**11.7819**	**1.1929**
D1 (stream)	1.786	0.0838	0.0893	0.0058	0.0055	**3.9689**	**7.3498**	0.7441
D2 (ditch)	6.086	0.2857	0.3043	0.0197	0.0187	**13.5244**	**25.0453**	**2.5358**
D2 (stream)	3.921	0.1841	0.1961	0.0127	0.0120	**8.7133**	**16.1358**	**1.6337**
D3 (ditch)	0.5689	0.0267	0.0284	0.0018	0.0017	**1.2642**	**2.3412**	0.237
D4 (pond)	0.2755	0.0129	0.0138	0.0009	0.0008	0.6122	**1.1337**	0.1147
D4 (stream)	0.4932	0.0232	0.0247	0.0016	0.0015	**1.0960**	**2.0296**	0.2055
D5 (pond)	0.4799	0.0225	0.0240	0.0016	0.0015	**1.0664**	**1.9749**	0.1999
D5 (stream)	0.6344	0.0298	0.0317	0.0021	0.0019	**1.4098**	**2.6107**	0.2643
D6 (ditch)	2.504	0.1176	0.1252	0.0081	0.0077	**5.5644**	**10.3045**	**1.0433**
R1 (pond)	0.04094	0.0019	0.0020	0.0001	0.0001	0.0910	0.1685	0.0170
R1 (stream)	2.289	0.1075	0.1145	0.0074	0.0070	**5.0867**	**9.4198**	0.9537
R3 (stream)	3.051	0.1432	0.1526	0.0099	0.0094	**6.7800**	**12.5556**	**1.2712**
R4 (stream)	0.8265	0.0388	0.0413	0.0027	0.0025	**1.8367**	**3.4012**	0.3443

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 0-13: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for flufenacet (120g flufenacet/ha) for each organism group based on FOCUS Steps 1, 2 and 3 calculations for the use of 2.0L/ha of GLOB1310aH in winter cereals at pre-emergence

Group		**Fish acute**	**Fish prolonged**	**Inverteb. acute**	**Inverteb. prolonged**	**Algae**	**Aquatic macro-phytes**	**Higher Tier**
Test species		*L. macrochirus*	*O. mykiss*	*D. magna*	*D. magna*	*P. subcapitata*	*L gibba*	*Microcosms*
Endpoint (µg/L)		LC_{50}	NOEC	LC_{50}	NOEC	EC_{50}	ErC_{50}	NOEC
		2130	200	30900	3260	4.5	2.43	12
AF		100	10	100	10	10	10	5
RAC (µg/L)		21.3	20	309	326	0.45	0.243	2.4
FOCUS Scenario	**PEC max (µg/L)**							
Step 1								
Worst-case	33.1207	**1.5550**	**1.6560**	0.1072	0.1016	**73.6016**	**136.2992**	0.3887
Step 2								
N-Europe	14.4272	0.6773	0.7214	0.0467	0.0443	**32.0604**	**59.3712**	0.1693
S-Europe	11.7207	0.5503	0.5860	0.0379	0.0360	**26.0460**	**48.2333**	0.1376
Step 3								
D1 (ditch)	3.928	0.184	0.196	0.013	0.012	**8.729**	**16.165**	**1.6366**
D1 (stream)	2.446	0.115	0.122	0.008	0.008	**5.436**	**10.066**	**1.0191**
D2 (ditch)	8.474	0.398	0.424	0.027	0.026	**18.831**	**34.872**	**3.5308**
D2 (stream)	5.449	0.256	0.272	0.018	0.017	**12.109**	**22.424**	**2.2704**
D3 (ditch)	0.7586	0.036	0.038	0.002	0.002	**1.686**	**3.122**	0.3160
D4 (pond)	0.3683	0.017	0.018	0.001	0.001	0.818	**1.516**	0.1534
D4 (stream)	0.6576	0.031	0.033	0.002	0.002	**1.461**	**2.706**	0.274
D5 (pond)	0.6399	0.030	0.032	0.002	0.002	**1.422**	**2.633**	0.2666
D5 (stream)	0.8515	0.040	0.043	0.003	0.003	**1.892**	**3.504**	0.35479
D6 (ditch)	3.309	0.155	0.165	0.011	0.010	**7.353**	**13.617**	**1.3787**
R1 (pond)	0.05526	0.003	0.003	0.000	0.000	0.123	0.227	0.0230
R1 (stream)	3.113	0.146	0.156	0.010	0.010	**6.918**	**12.811**	**1.2970**
R3 (stream)	4.155	0.195	0.208	0.013	0.013	**9.233**	**17.099**	**1.7312**
R4 (stream)	1.088	0.051	0.054	0.004	0.003	**2.418**	**4.477**	0.45333

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

For the intended uses, calculated PEC/RAC ratios did not indicate an acceptable risk for the most sensitive group of aquatic organisms (risk for primary producers as characterised by a microcosms NOEC of 12 µg/L in connection with an assessment factor of 5) in several FOCUS Steps 1-3 scenarios. Therefore, further PEC/RAC ratios were calculated based on FOCUS Step 4 PEC_{SW} considering reduced exposure of surface water bodies according to the calculations based on standard Landscape and Mitigation Report.

As reported in the Part B section 8.9, depending on the scenario the main entrance of flufenacet is via drift, run-off or drainage. For drift and run-off, either a no spray buffer zone of drift reduction nozzles or vegetated buffer strip or a combination of these can be applied. However , no refinement was possible for drainage.

Table 0-14: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Flufenacet based on FOCUS Step 4 calculations and toxicity data for 1.5L/ha with mitigation of spray drift and run-off for the use of GLOB1310aH in winter cereals at pre-emergence (90g Flufenacet/ha pre-emergence)

PEC_{sw} (µg/L)	**Scenario**			**STEP 4 Flufenacet**						
Nozzle	Vegetative strip (m)	None	None	None	None	None	10	10	20	20
reduction	No spray buffer (m)	1/mrt	5	10	15	20	10	50	20	50
None	D1 ditch	-	2.863	2.863	-	-	2.863	2.863	2.863	2.863
50%		2.863	2.863		-	-	2.863	-	2.863	-
75%		2.863	2.863		-	-	2.863	-	2.863	-
90%		2.863	2.863		-	-	2.863	-	2.863	-
None	D1 stream	-	1.786	1.786	-	-	1.786	1.786	1.786	1.786
50%		1.786	1.786		-	-	1.786	-	1.786	-
75%		1.786	1.786		-	-	1.786	-	1.786	-
90%		1.786	1.786		-	-	1.786	-	1.786	-
None	D2 ditch	-	6.086	6.086	-	-	6.086	6.086	6.086	6.086
50%		6.086	6.086		-	-	6.086	-	6.086	-
75%		6.086	6.086		-	-	6.086	-	6.086	-
90%		6.086	6.086		-	-	6.086	-	6.086	-
None	D2 stream	-	3.921	3.921	-	-	3.921	3.921	3.921	3.921
50%		3.921	3.921		-	-	3.921	-	3.921	-
75%		3.921	3.921		-	-	3.921	-	3.921	-
90%		3.921	3.921		-	-	3.921	-	3.921	-
None	D3 ditch	-	0.1542	0.08181	-	-	0.08181	0.0176	0.04251	0.0176
50%		0.3461	0.07712	-	-	-	0.0409	-	0.02125	-
75%		0.1731	0.03854	-	-	-	0.02045	-	0.01062	-
90%		0.06924	0.01542	-	-	-	0.00818	-	0.00425	-
None	D4 pond	-	0.2749	0.2737	-	-	0.2737	0.2717	0.2727	0.2717
50%		0.2746	0.2728	-	-	-	0.2722	-	0.2717	-
75%		0.2726	0.2717	-	-	-	0.2714	-	0.2712	-
90%		0.2715	0.2711	-	-	-	0.271	-	0.2709	-
None	D4 stream	-	0.3269	0.3269	-	-	0.3269	0.3269	0.3269	0.3269
50%		0.4043	0.3269	-	-	-	0.3269	-	0.3269	-
75%		0.3269	0.3269	-	-	-	0.3269	-	0.3269	-

90%		0.3269	0.3269	-	-	-	0.3269	-	0.3269	-
None	D5 pond	-	0.4792	0.478	-	-	0.478	0.4759	0.4769	0.4759
50%		0.4789	0.477	-	-	-	0.4764	-	0.4759	-
75%		0.4769	0.4759	-	-	-	0.4756	-	0.4753	-
90%		0.4756	0.4753	-	-	-	0.4751	-	0.475	-
None	D5 stream	-	0.6344	0.6344	-	-	0.6344	0.6344	0.6344	0.6344
50%		0.6344	0.6344	-	-	-	0.6344	-	0.6344	-
75%		0.6344	0.6344	-	-	-	0.6344	-	0.6344	-
90%		0.6344	0.6344	-	-	-	0.6344	-	0.6344	-
None	D6 ditch	-	2.504	2.504	-	-	2.504	2.504	2.504	2.504
50%		2.504	2.504	-	-	-	2.504	-	2.504	-
75%		2.504	2.504	-	-	-	2.504	-	2.504	-
90%		2.504	2.504	-	-	-	2.504	-	2.504	-
None	R1 pond	-	0.03872	0.03474	-	-	0.02001	0.01336	0.0117	0.008443
50%		0.03778	0.03164	-	-	-	0.01491	-	0.008301	-
75%		0.03119	0.02932	-	-	-	0.0126	-	0.00607	-
90%		0.02878	0.02803	-	-	-	0.01165	-	0.00595	-
None	R1 stream	-	-	-	-	-	1.026	1.026	0.5342	0.5342
50%		-	-	-	-	-	1.026	-	0.5342	-
75%		-	-	-	-	-	1.026	-	0.5342	-
90%		-	-	-	-	-	1.026	-	0.5342	-
None	R3 stream	-	-	-	-	-	1.392	0.7303	0.7303	0.7303
50%		-	-	-	-	-	0.7303	-	0.7303	-
75%		-	-	-	-	-	0.7303	-	0.7303	-
90%		-	-	-	-	-	0.7303	-	0.7303	-
None	R4 stream	-	-	-	-	-	0.3731	0.1949	0.1949	0.1949
50%		-	-	-	-	-	0.1949	-	0.1949	-
75%		-	-	-	-	-	0.1949	-	0.1949	-
90%		-	-	-	-	-	0.1949	-	0.1949	-
RAC (µg/L)										
2.4 (primary producers)		**PEC/RAC ratio**								
None	D1 Ditch	-	**1.19292**	**1.19292**	-	-	**1.19292**	**1.1929**	**1.1929**	**1.1929**
50%		**1.19292**	**1.19292**	-	-	-	**1.19292**	-	**1.1929**	-
75%		**1.19292**	**1.19292**	-	-	-	**1.19292**	-	**1.1929**	-
90%		**1.19292**	**1.19292**	-	-	-	**1.19292**	-	**1.1929**	-
None	D1 stream	-	0.74417	0.74417	-	-	0.74417	0.7442	0.7442	0.7442
50%		0.74417	0.74417	-	-	-	0.74417	-	0.7442	-
75%		0.74417	0.74417	-	-	-	0.74417	-	0.7442	-
90%		0.74417	0.74417	-	-	-	0.74417	-	0.7442	-
None	D2 Ditch	-	**2.53583**	**2.53583**	-	-	**2.53583**	**2.5358**	**2.5358**	**2.5358**
50%		**2.53583**	**2.53583**	-	-	-	**2.53583**	-	**2.5358**	-
75%		**2.53583**	**2.53583**	-	-	-	**2.53583**	-	**2.5358**	-
90%		**2.53583**	**2.53583**	-	-	-	**2.53583**	-	**2.5358**	-

None	D2 Stream	-	**1.63375**	**1.63375**	-	-	**1.63375**	**1.6338**	**1.6338**	**1.6338**
50%		**1.63375**	**1.63375**	-	-	-	**1.63375**	-	**1.6338**	-
75%		**1.63375**	**1.63375**	-	-	-	**1.63375**	-	**1.6338**	-
90%		**1.63375**	**1.63375**	-	-	-	**1.63375**	-	**1.6338**	-
None	D3 Ditch	-	0.06425	0.03409	-	-	0.03409	0.0073	0.0177	0.0073
50%		0.14421	0.03213	-	-	-	0.01704	-	0.0089	-
75%		0.07213	0.01606	-	-	-	0.00852	-	0.0044	-
90%		0.02885	0.00643	-	-	-	0.00341	-	0.0018	-
None	D4 Pond	-	0.11454	0.11404	-	-	0.11404	0.1132	0.1136	0.1132
50%		0.11442	0.11367	-	-	-	0.11342	-	0.1132	-
75%		0.11358	0.11321	-	-	-	0.11308	-	0.1130	-
90%		0.11313	0.11296	-	-	-	0.11292	-	0.1129	-
None	D4 Stream	-	0.13621	0.13621	-	-	0.13621	0.1362	0.1362	0.1362
50%		0.16846	0.13621	-	-	-	0.13621	-	0.1362	-
75%		0.13621	0.13621	-	-	-	0.13621	-	0.1362	-
90%		0.13621	0.13621	-	-	-	0.13621	-	0.1362	-
None	D5 Pond	-	0.19967	0.19917	-	-	0.19917	0.1983	0.1987	0.1983
50%		0.19954	0.19875	-	-	-	0.19850	-	0.1983	-
75%		0.19871	0.19829	-	-	-	0.19817	-	0.1980	-
90%		0.19817	0.19804	-	-	-	0.19796	-	0.1979	-
None	D5 Stream	-	0.26433	0.26433	-	-	0.26433	0.2643	0.2643	0.2643
50%		0.26433	0.26433	-	-	-	0.26433	-	0.2643	-
75%		0.26433	0.26433	-	-	-	0.26433	-	0.2643	-
90%		0.26433	0.26433	-	-	-	0.26433	-	0.2643	-
None	D6 Ditch	-	**1.04333**	**1.04333**	-	-	**1.04333**	**1.0433**	**1.0433**	**1.0433**
50%		**1.04333**	**1.04333**	-	-	-	**1.04333**	-	**1.0433**	-
75%		**1.04333**	**1.04333**	-	-	-	**1.04333**	-	**1.0433**	-
90%		**1.04333**	**1.04333**	-	-	-	**1.04333**	-	**1.0433**	-
None	R1 Pond	-	0.01613	0.01448	-	-	0.00834	0.0056	0.0049	0.0035
50%		0.01574	0.01318	-	-	-	0.00621	-	0.0035	-
75%		0.01300	0.01222	-	-	-	0.00525	-	0.0025	-
90%		0.01199	0.01168	-	-	-	0.00485	-	0.0025	-
None	R1 stream	-	-	-	-	-	0.42750	0.4275	0.2226	0.2226
50%		-	-	-	-	-	0.42750	-	0.2226	-
75%		-	-	-	-	-	0.42750	-	0.2226	-
90%		-	-	-	-	-	0.42750	-	0.2226	-
None	R3 stream	-	-	-	-	-	0.58000	0.5800	0.3043	0.3043
50%		-	-	-	-	-	0.30429	-	0.3043	-
75%		-	-	-	-	-	0.30429	-	0.3043	-
90%		-	-	-	-	-	0.30429	-	0.3043	-
None	R4 stream	-	-	-	-	-	0.15546	0.0812	0.0812	0.0812
50%		-	-	-	-	-	0.08121	-	0.0812	-
75%		-	-	-	-	-	0.08121	-	0.0812	-

90%		-	-	-	-	-	0.08121	-	0.0812	-

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold; Np: not performed

Table 0-15: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for Flufenacet based on FOCUS Step 4 calculations and toxicity data for 2.0L/ha with mitigation of spray drift and run-off for the use of GLOB1310aH in winter cereals at pre-emergence (120g Flufenacet/ha pre-emergence)

PEC_{sw} (µg/L)	**Scenario**	**STEP 4 Flufenacet**						
Nozzle	Vegetative strip (m)	None	None	None	10	10	20	20
reduction	No spray buffer (m)	1/mrt	5	10	10	50	20	50
None	D1 ditch	3.928	3.928	3.928	3.928	3.928	3.928	3.928
50%		3.928	3.928	3.928	3.928	-	3.928	-
75%		3.928	3.928	3.928	3.928	-	3.928	-
90%		3.928	3.928	3.928	3.928	-	3.928	-
None	D1 stream	2.446	2.446	2.446	2.446	2.446	2.446	2.446
50%		2.446	2.446	2.446	2.446	-	2.446	-
75%		2.446	2.446	2.446	2.446	-	2.446	-
90%		2.446	2.446	2.446	2.446	-	2.446	-
None	D2 ditch	8.474	8.474	8.474	8.474	8.474	8.474	8.474
50%		8.474	8.474	8.474	8.474	-	8.474	-
75%		8.474	8.474	8.474	8.474	-	8.474	-
90%		8.474	8.474	8.474	8.474	-	8.474	-
None	D2 stream	5.449	5.449	5.449	5.449	5.449	5.449	5.449
50%		5.449	5.449	5.449	5.449	-	5.449	-
75%		5.449	5.449	5.449	5.449	-	5.449	-
90%		5.449	5.449	5.449	5.449	-	5.449	-
None	D3 ditch		0.2056	0.1091	0.1091	0.02346	0.05668	0.02346
50%		0.4615	0.1028	0.5451	0.05451	-	0.02833	-
75%		0.2308	0.0514	0.02726	0.02726	-	0.01417	-
90%		0.0923	0.02056	0.01091	0.010914	-	0.005667	-
None	D4 pond	-	0.3675	0.3659	0.3659	0.3633	0.3646	0.3633
50%		0.3671	0.347	0.3639	0.3639	-	0.3632	-
75%		0.3645	0.3633	0.3629	0.3629	-	0.3626	-
90%		0.3629	0.3625	0.3623	0.3623	-	0.3622	-
None	D4 stream	0.4432	0.4432	0.4432	0.4432	0.4432	0.4432	0.4432
50%		0.4432	0.4432	0.4432	0.4432	-	0.4432	-
75%		0.4432	0.4432	0.4432	0.4432	-	0.4432	-
90%		0.4432	0.4432	0.4432	0.4432	-	0.4432	-
None	D5 pond	-	0.639	0.6373	0.6373	0.6346	0.6359	0.6346
50%		0.6386	0.636	0.6352	0.6352	-	0.6345	-
75%		0.6358	0.6346	0.6342	0.6342	-	0.6338	-
90%		0.6342	0.6337	0.6335	0.6335	-	0.6334	-

None	D5 stream	0.8515	0.8515	0.8515	0.8515	0.8515	0.8515	0.8515
50%		0.8515	0.8515	0.8515	0.8515	-	0.8515	-
75%		0.8515	0.8515	0.8515	0.8515	-	0.8515	-
90%		0.8515	0.8515	0.8515	0.8515	-	0.8515	-
None	D6 ditch	3.309	3.309	3.309	3.309	3.309	3.309	3.309
50%		3.309	3.309	3.309	3.309	-	3.309	-
75%		3.309	3.309	3.309	3.309	-	3.309	-
90%		3.309	3.309	3.309	3.309	-	3.309	-
None	R1 pond	-	0.05231	0.04699	0.02695	0.01819	0.01574	0.01139
50%		0.05105	0.04285	0.0402	0.02015	-	0.01121	-
75%		0.04222	0.03815	0.03717	0.01676	-	0.008944	-
90%		0.03725	0.03625	0.03593	0.01509	-	0.0077	-
None	R1 stream	-	-	-	1.395	1.395	0.7266	0.7266
50%		-	-	-	1.395	-	0.7266	-
75%		-	-	-	1.395	-	0.7266	-
90%		-	-	-	1.395	-	0.7266	-
None	R3 stream	-	-	-	1.896	1.896	0.9946	0.9946
50%		-	-	-	1.896	-	0.9946	-
75%		-	-	-	1.896	-	0.9946	-
90%		-	-	-	1.896	-	0.9946	-
None	R4 stream	-	-	-	0.4911	0.4911	0.2565	0.2565
50%		-	-	-	0.4911	-	0.2565	-
75%		-	-	-	0.4911	-	0.2565	-
90%		-	-	-	0.4911	-	0.2565	-
RAC (µg/L)								
2.4 (primry producers)		**PEC/RAC ratio**						
None	D1 Ditch	**1.6367**	**1.6367**	**1.6367**	**1.63667**	**1.6367**	**1.6367**	**1.6367**
50%		**1.6367**	**1.6367**	**1.6367**	**1.63667**	-	**1.6367**	-
75%		**1.6367**	**1.6367**	**1.6367**	**1.63667**	-	**1.6367**	-
90%		**1.6367**	**1.6367**	**1.6367**	**1.63667**	-	**1.6367**	-
None	D1 stream	**1.0192**	**1.0192**	**1.0192**	**1.01917**	**1.0192**	**1.0192**	**1.0192**
50%		**1.0192**	**1.0192**	**1.0192**	**1.01917**	-	**1.0192**	-
75%		**1.0192**	**1.0192**	**1.0192**	**1.01917**	-	**1.0192**	-
90%		**1.0192**	**1.0192**	**1.0192**	**1.01917**	-	**1.0192**	-
None	D2 Ditch	**3.5308**	**3.5308**	**3.5308**	**3.53083**	**3.5308**	**3.5308**	**3.5308**
50%		**3.5308**	**3.5308**	**3.5308**	**3.53083**	-	**3.5308**	-
75%		**3.5308**	**3.5308**	**3.5308**	**3.53083**	-	**3.5308**	-
90%		**3.5308**	**3.5308**	**3.5308**	**3.53083**	-	**3.5308**	-
None	D2 Stream	**2.2704**	**2.2704**	**2.2704**	**2.27042**	**2.2704**	**2.2704**	**2.2704**
50%		**2.2704**	**2.2704**	**2.2704**	**2.27042**	-	**2.2704**	-
75%		**2.2704**	**2.2704**	**2.2704**	**2.27042**	-	**2.2704**	-
90%		**2.2704**	**2.2704**	**2.2704**	**2.27042**	-	**2.2704**	-
None	D3 Ditch	0.0000	0.0857	0.0455	0.04546	0.0098	0.0236	0.0098

50%		0.1923	0.0428	0.2271	0.02271	-	0.0118	-
75%		0.0962	0.0214	0.0114	0.01136	-	0.0059	-
90%		0.0385	0.0086	0.0045	0.00455	-	0.0024	-
None	D4 Pond	-	0.1531	0.1525	0.15246	0.1514	0.1519	0.1514
50%		0.1530	0.1446	0.1516	0.15163	-	0.1513	-
75%		0.1519	0.1514	0.1512	0.15121	-	0.1511	-
90%		0.1512	0.1510	0.1510	0.15096	-	0.1509	-
None	D4 Stream	0.1847	0.1847	0.1847	0.18467	0.1847	0.1847	0.1847
50%		0.1847	0.1847	0.1847	0.18467	-	0.1847	-
75%		0.1847	0.1847	0.1847	0.18467	-	0.1847	-
90%		0.1847	0.1847	0.1847	0.18467	-	0.1847	-
None	D5 Pond	-	0.2663	0.2655	0.26554	0.2644	0.2650	0.2644
50%		0.2661	0.2650	0.2647	0.26467	-	0.2644	-
75%		0.2649	0.2644	0.2643	0.26425	-	0.2641	-
90%		0.2643	0.2640	0.2640	0.26396	-	0.2639	-
None	D5 Stream	0.3548	0.3548	0.3548	0.35479	0.3548	0.3548	0.3548
50%		0.3548	0.3548	0.3548	0.35479	-	0.3548	-
75%		0.3548	0.3548	0.3548	0.35479	-	0.3548	-
90%		0.3548	0.3548	0.3548	0.35479	-	0.3548	-
None	D6 Ditch	**1.3788**	**1.3788**	**1.3788**	**1.37875**	**1.3788**	**1.3788**	**1.3788**
50%		**1.3788**	**1.3788**	**1.3788**	**1.37875**	-	**1.3788**	-
75%		**1.3788**	**1.3788**	**1.3788**	**1.37875**	-	**1.3788**	-
90%		**1.3788**	**1.3788**	**1.3788**	**1.37875**	-	**1.3788**	-
None	R1 Pond	-	0.0218	0.0196	0.01123	0.0076	0.0066	0.0047
50%		0.0213	0.0179	0.0168	0.00840	-	0.0047	-
75%		0.0176	0.0159	0.0155	0.00698	-	0.0037	-
90%		0.0155	0.0151	0.0150	0.00629	-	0.0032	-
None	R1 stream	-	-	-	0.58125	0.5813	0.3028	0.3028
50%		-	-	-	0.58125	-	0.3028	-
75%		-	-	-	0.58125	-	0.3028	-
90%		-	-	-	0.58125	-	0.3028	-
None	R3 stream	-	-	-	0.79000	0.7900	0.4144	0.4144
50%		-	-	-	0.79000	-	0.4144	-
75%		-	-	-	0.79000	-	0.4144	-
90%		-	-	-	0.79000	-	0.4144	-
None	R4 stream	-	-	-	0.20463	0.2046	0.1069	0.1069
50%		-	-	-	0.20463	-	0.1069	-
75%		-	-	-	0.20463	-	0.1069	-
90%		-	-	-	0.20463	-	0.1069	-

PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold; Np: not performed

zRMS comments:
Flufenacet
Based on the PEC FOCUS Step 4 values the risk is acceptable for scenarios D3 Ditch, D4 Pond, D4

Stream, D5 Pond, D5 Stream, , R1 Pond, R1 Stream, R3 Stream and R4 Stream when the appropriate risk mitigation measures have been applied. In the case of D1 Ditch, D1 Stream, D2 Ditch, D2 Stream and D6 Ditch the risk is unacceptable.

Flufenacet metabolites

As worst case, only the highest intended application rate (2L GLOB1310aH/ha at pre-emergence) was used for the flufenacet metabolites risk assessment. This risk assessment covers all other intended uses.

Table 0-16: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for FOE-sulfonic acid (M2) for each organism group based on FOCUS Steps 1 and 2 calculations for the use of 2.0L/ha of GLOB1310aH in winter cereals at pre-emergence

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic mac-rophytes**
Test species		*O. mykiss*	*D. magna*	*P. subcapitata*	*L gibba*
Endpoint (µg/L)		LC_{50}	LC_{50}	EC_{50}	ErC_{50}
		86700	87300	86700	86700
AF		100	100	10	10
RAC (µg/L)		867	873	8670	8670
FOCUS Scenario	**PEC max (µg/L)**				
Step 1					
Worst-case	8.8447	0.0102	0.0101	0.0010	0.0010
Step 2					
N-Europe	4.2828	0.0049	0.0049	0.0005	0.0005
S-Europe	3.4313	0.0040	0.0039	0.0004	0.0004

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 0-17: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for FOE-oxalate (M1) for each organism group based on FOCUS Steps 1 and 2 calculations for the use of 2.0L/ha of GLOB1310aH in winter cereals at pre-emergence

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic mac-rophytes**
Test species		*O. mykiss*	*D. magna*	*P. subcapitata*	*L gibba*
Endpoint (µg/L)		LC_{50}	LC_{50}	EC_{50}	ErC_{50}
		86700	87300	86700	86700
AF		100	100	10	10
RAC (µg/L)		867	873	8670	8670
FOCUS Scenario	**PEC max (µg/L)**				
Step 1					
Worst-case	7.2345	0.0083	0.0083	0.0008	0.0008
Step 2					
N-Europe	2.4634	0.0028	0.0028	0.0003	0.0003
S-Europe	1.9748	0.0023	0.0023	0.0002	0.0002

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 0-18: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Thiadone (M9) for each organism group based on FOCUS Steps 1 and 2 calculations for the use of 2.0L/ha of GLOB1310aH in winter cereals at pre-emergence

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		*O. mykiss*	*D. magna*	*P. subcapitata*
Endpoint (µg/L)		LC_{50}	LC_{50}	EC_{50}
		9100	31700	4100
AF		100	100	10
RAC (µg/L)		91	317	410
FOCUS Scenario	**PEC max (µg/L)**			
Step 1				
Worst-case	5.5381	0.060858242	0.017470347	0.013507561
Step 2				
N-Europe	2.7232	0.029925275	0.008590536	0.006641951
S-Europe	2.1817	0.023974725	0.006882334	0.00532122

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 0-19: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for Methylsulfide (M5) for each organism group based on FOCUS Steps 1 and 2 calculations for the use of 2.0L/ha of GLOB1310aH in winter cereals at pre-emergence

Group		**Fish acute**	**Inverteb. acute**	**Algae**	**Aquatic mac-rophytes**
Test species		*O. mykiss*	*D. magna*	*P. subcapitata*	*L gibba*
Endpoint (µg/L)		LC_{50}	LC_{50}	EC_{50}	ErC_{50}
		86700	87300	83300	86700
AF		100	100	10	10
RAC (µg/L)		867	873	8330	8670
FOCUS Scenario	**PEC max (µg/L)**				
Step 1					
Worst-case	7.8511	0.0091	0.0090	0.0009	0.0009
Step 2					
N-Europe	3.8654	0.0045	0.0045	0.0045	0.0045
S-Europe	3.0928	0.0036	0.0036	0.0036	0.0036

AF: Assessment factor; PEC: Predicted environmental concentration; RAC: Regulatory acceptable concentration; PEC/RAC ratios above the relevant trigger of 1 are shown in bold

zRMS comments: the endpoints relevant for the risk assessment have been established on the EU level for the following metabolites of flufenacet (SANCO/7469/VI/98-Final, 03 July 2003):
- fish: FOE-sulfonic acid, Thiadone
- invertebrates: FOE-sulfonic acid, Thiadone,

- algae: FOE-sulfonic acid, Thiadone, FOE-methylsulfide,
- aquatic plants: FOE-sulfonic acid,
and only these endpoint were considered in the risk assessment by zRMS. The risk is acceptable.

9.5.2.3 Formulation GLOB1310aH

The Regulation (EC) No 1107/2009, in Article 29, requires that 'interaction between the a.s., safeners, synergists and co-formulants shall be taken into account in the evaluation and authorisation.

Two approaches for the combined risk assessment are presented here. The first risk assessment is based on the comparison of the measured toxicity of the formulation and the PEC drift only (from Swash drift calculator). This approach has been commonly accepted in different countries so we provide it here for completeness sake. The second one is a stepwise approach according to the decision scheme provided in the Guidance EFSA (2013[1]) to perform the risk assessment for formulations containing more than one active substance. A tool called AGD_AquaMix_v1.15 is implementing the different steps of the Scheme.

Formulation GLOB1310aH – drift PECsw formulation

It was not necessary to calculate an LC_{50} based on the toxicity of the mixture or a MDR for fish. Considering of the chronic toxicity, the risk assessment of Aclonifen is driven by the lowest fish endpoint (i.e. $NOEC_{growth}$ = 5 µg/L). According to the data requirements for product evaluations, formulation studies on the long-term toxicity are only triggered when '*it is not possible to extrapolate from data obtained in the corresponding studies on the active substance (for example the plant protection product is more acutely toxic than the active substance as manufactured by a factor of 10)*' (Commission Regulation (EU) No 284/2013). In the current situation, the toxicity of the formulated product GLOB1310aH is not higher than the one of the active substances. In addition, it is generally observed that chronic effects are mainly driven by the active ingredient (and not the co-formulant), as the product is quickly under its active ingredient form in the environment. We also refer to the analysis of the predicted toxicity versus observed toxicity (below), that did not show any synergistic effect of both active ingredients in the formulation GLOB1310aH on algae, Daphnia and macrophytes). From the combined risk assessment it is clear that the toxicity driver of the toxicity to fish is Aclonifen. Therefore, the applicant considers that the risk assessment is covered by the respective risk assessment of each active ingredient for chronic fish and that a vertebrate's study on fish was not necessary since it would not have change the outcome of risk assessment.

Measured toxicity data (ECx) on the active substances and on the formulation are available for specific endpoints. As the endpoint of the actives substances on *Myriophyllum* is not available, the combined risk assessment is performed for Daphnia, algae and *Lemna*. In the case of *Myriophyllum* the measured endpoint is used for the risk assessment.

Table 0-20: MDR calculation for GLOB1310aH

Organism	Fraction in mixture		LC50 /EC50 (mg as/L)		Predicted Ecmix LC/EC50	Measured EC/LC50	MDR
	Aclonifen	Flufenacet	Aclonifen	Flufenacet	(mg as/L)	Studies (mg as/L)	
Daphnia	0.9	0.1	1.2	30.9	1.328	3.017	0.44
Algae*	0.9	0.1	0.0069	0.0045	0.007	0.02662	0.25
Lemna	0.9	0.1	0.012	0.00243	0.009	0.0057	1.51

[1] Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Panel on Plant Protection Products and their Residues (PPR). Scientific opinion, EFSA Journal 2013;11(7):3290.

*The agreed endpoint for aclonifen is set for *Desmodesmus subspicatus* (most sensitive species for Aclonifen); while, for flufenacet the algal species whit an agreed endpoint is *P. subcapitata* (*P. subcapiata* was confirmed as the most sensitive algal species in the RAR flufenacet). As the endpoint for algae is established in two different algal species (with different sensitivity) for aclonifen and flufenacet, a comparison is not suitable. However, here in order to obtain a MDR value the endpoint of the two different algal species is used.

Following this approach, the calculated MDR values are between 0.2 and 5 for the aquatic organisms under consideration, indicating that the formulation does not cause an increased toxicity compared to the active substances. No synergisms or additional toxicity occurs due to the co-formulants or combination of active substances. Therefore, the risk assessments presented for the active substances also cover the risk to aquatic organisms following the proposed uses of GLOB1310aH and no additional TER calculations is needed for the formulated product.

However, for completeness and transparency sake, and because some countries may find this approach acceptable, an assessment of the toxicity driver was conducted and the formulation PEC/RAC calculations are presented below.

Table 0-21: Identification of a toxicity driver for aquatic organisms

Organism	Active	EC (mg/L)	Fraction in mixture	Toxic unit	Tox of the sum ai	Contribution to toxicity	Conclusion
Fish	Aclonifen	0.67	0.9	1.3433	0.719	96.623	Aclonifen is the toxicity driver
	Flufenacet	2.13	0.1	0.0469		3.377	
Daphnia	Aclonifen	0.952	0.9	0.9454	1.054	99.659	Aclonifen is the toxicity driver
	Flufenacet	30.9	0.1	0.0032		0.341	
Algae	Aclonifen	0.0069	0.9	130.4348	0.007	85.443	No clear toxicity driver was identified
	Flufenacet	0.0045	0.1	22.2222		14.557	
Lemna	Aclonifen	0.012	0.9	75.0000	0.009	64.570	No clear toxicity driver was identified
	Flufenacet	0.00243	0.1	41.1523		35.430	

Aclonifen is the toxicity driver for fish and Daphnia, thus reference is made to the risk assessment of the active substance aclonifen. The risk mitigation measures needed for the active substance aclonifen will be protective for fish and Daphnia. No clear identification of toxicity driver was possible for algae an Lemna, thus reference is made to the risk assessment where the endpoint from the studies with the formulation is used. Additionally, for Myriophyllum the risk assessment based on experimental data are presented in the tables below.

Table 0-22: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for GLOB1310aH based on drift calculations and toxicity data for 1.5L/ha for the use of GLOB1310aH in winter cereals at pre-emergence

Group		Inverteb. acute	Algae	Algae	Aquatic macrophytes	Aquatic macrophytes
Test species		*D. magna*	*D. subcapitatum*	*P. subcapitata*	*L. gibba*	*M. spicatum*
Endpoint (µg/L)		EC50	ErC50 mm	ErC50	ErC50	ErC50
		6120	103.8	54.2	11.6	301.9
AF		100	10	10	100	10
RAC (µg/L)		61.2	10.38	5.42	0.116	30.19
FOCUS Scenario	**PEC max (µg/L)**					
1 m no spray buffer						
Ditch	10.8101	0.0177	**1.0414**	**1.9945**	**9.3191**	0.3581
Pond	0.3686	0.0006	0.0355	0.0680	0.3178	0.0122
Stream*	9.6269	0.0157	0.9274	**1.7762**	**8.2991**	0.3189
3 m no spray buffer						
Ditch	4.5767	0.0075	0.4409	0.8444	**3.9454**	0.1516
Pond	0.3906	0.0006	0.0376	0.0721	0.3367	0.0129

Stream*	5.492	0.0090	0.5291	**1.0133**	**4.7345**	0.1819
5 m no spray buffer						
Ditch	2.9302	0.0048	0.2823	0.5406	**2.5260**	0.0971
Pond	0.3189	0.0005	0.0307	0.0588	0.2749	0.0106
Stream*	3.5162	0.0057	0.3387	0.6487	**3.0312**	0.1165
5 m no spray buffer +50% drift reduction nozzles						
Ditch	1.4651	0.0024	0.1411	0.2703	**1.2630**	0.0485
Pond	0.15945	0.0003	0.0154	0.0294	0.1375	0.0053
Stream*	1.7581	0.0029	0.1694	0.3244	**1.5156**	0.0582
5 m no spray buffer +75% drift reduction nozzles						
Ditch	0.73255	0.0012	0.0706	0.1352	0.6315	0.0243
Pond	0.079725	0.0001	0.0077	0.0147	0.0687	0.0026
Stream*	0.87905	0.0014	0.0847	0.1622	0.7578	0.0291
5 m no spray buffer +90% drift reduction nozzles						
Ditch	0.29302	0.0005	0.0282	0.0541	0.2526	0.0097
Pond	0.03189	0.0001	0.0031	0.0059	0.0275	0.0011
Stream*	0.35162	0.0006	0.0339	0.0649	0.3031	0.0116
10 m no spray buffer						
Ditch	1.554	0.0025	0.1497	0.2867	**1.3397**	0.0515
Pond	0.2293	0.0004	0.0221	0.0423	0.1977	0.0076
Stream*	1.8648	0.0030	0.1797	0.3441	**1.6076**	0.0618
10 m no spray buffer + 50% drift reduction nozzles						
Ditch	0.777	0.0013	0.0749	0.1434	0.6698	0.0257
Pond	0.11465	0.0002	0.0110	0.0212	0.0988	0.0038
Stream*	0.9324	0.0015	0.0898	0.1720	0.8038	0.0309
10 m no spray buffer + 75% drift reduction nozzles						
Ditch	0.3885	0.0006	0.0374	0.0717	0.3349	0.0129
Pond	0.057325	0.0001	0.0055	0.0106	0.0494	0.0019
Stream*	0.4662	0.0008	0.0449	0.0860	0.4019	0.0154
10 m no spray buffer + 90% drift reduction nozzles						
Ditch	0.1554	0.0003	0.0150	0.0287	0.1340	0.0051
Pond	0.02293	0.00004	0.0022	0.0042	0.0198	0.0008
Stream*	0.18648	0.0003	0.0180	0.0344	0.1608	0.0062
20 m no spray buffer						
Ditch	0.8074	0.0013	0.0778	0.1490	0.6960	0.0267
Pond	0.1531	0.00025	0.0147	0.0282	0.1320	0.0051
Stream*	0.9689	0.0016	0.0933	0.1788	0.8353	0.0321
20 m no spray buffer + 50% drift reduction nozzles						
Ditch	0.4037	0.0007	0.0389	0.0745	0.3480	0.0134
Pond	0.07655	0.0001	0.0074	0.0141	0.0660	0.0025
Stream*	0.48445	0.0008	0.0467	0.0894	0.4176	0.0160
20 m no spray buffer + 75% drift reduction nozzles						
Ditch	0.20185	0.0003	0.0194	0.0372	0.1740	0.0067
Pond	0.038275	0.0001	0.0037	0.0071	0.0330	0.0013
Stream*	0.242225	0.0004	0.0233	0.0447	0.2088	0.0080
20 m no spray buffer + 90% drift reduction nozzles						
Ditch	0.08074	0.0001	0.0078	0.0149	0.0696	0.0027
Pond	0.01531	0.00003	0.0015	0.0028	0.0132	0.0005
Stream*	0.09689	0.0002	0.0093	0.0179	0.0835	0.0032

PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Table 0-23: Aquatic organisms: PEC calculation and acceptability of risk (PEC/RAC < 1) for GLOB1310aH based on drift calculations and toxicity data for 2.0L/ha for the use of GLOB1310aH in winter cereals at pre-emergence

Group		**Inverteb. acute**	**Algae**	**Algae**	**Aquatic macrophytes**	**Aquatic macrophytes**
Test species		*D. magna*	*D. subspicatus*	*P. subcapitata*	*L. gibba*	*M. spicatum*
Endpoint (µg/L)		EC50	ErC50 mm	ErC50	ErC50	ErC50
		6120	103.8	54.2	11.6	301.9
AF		10	10	10	10	10
RAC (µg/L)		612	10.38	5.42	1.16	30.19

FOCUS Scenario	PEC max (µg/L)					
1 m no spray buffer						
Ditch	15.7815	0.0258	**1.5204**	**2.9117**	**13.6047**	0.5227
Pond	0.8062	0.0013	0.0777	0.1487	0.6950	0.0267
Stream*	18.9378	0.0309	**1.8245**	**3.4941**	**16.3257**	0.6273
3 m no spray buffer						
Ditch	6.6814	0.0109	0.6437	**1.2327**	**5.7598**	0.2213
Pond	~~0.5792~~ 0.5702	0.0009	~~0.0558~~ 0.0549	~~0.1069~~ 0.1052	~~0.4993~~ 0.4916	~~0.0192~~ 0.0189
Stream*	8.0177	0.0131	0.7724	**1.4793**	**6.9118**	0.2656
5 m no spray buffer						
Ditch	4.2777	0.0070	0.4121	0.7892	**3.6877**	0.1417
Pond	0.4656	0.0008	0.0449	0.0859	0.4014	0.0154
Stream*	5.1332	0.0084	0.4945	0.9471	**4.4252**	0.1700
5 m no spray buffer +50% drift reduction nozzles						
Ditch	2.13885	0.0035	0.2061	0.3946	**1.8438**	0.0708
Pond	0.2328	0.0004	0.0224	0.0430	0.2007	0.0077
Stream*	2.5666	0.0042	0.2473	0.4735	**2.2126**	0.0850
5 m no spray buffer +75% drift reduction nozzles						
Ditch	1.069425	0.0017	0.1030	0.1973	0.9219	0.0354
Pond	0.1164	0.0002	0.0112	0.0215	0.1003	0.0039
Stream*	1.2833	0.0021	0.1236	0.2368	**1.1063**	0.0425
5 m no spray buffer +90% drift reduction nozzles						
Ditch	0.42777	0.0007	0.0412	0.0789	0.3688	0.0142
Pond	0.04656	0.0001	0.0045	0.0086	0.0401	0.0015
Stream*	0.51332	0.0008	0.0495	0.0947	0.4425	0.0170
10 m no spray buffer						
Ditch	2.2687	0.0037	0.2186	0.4186	**1.9558**	0.0751
Pond	0.3348	0.0005	0.0323	0.0618	0.2886	0.0111
Stream*	2.7224	0.0044	0.2623	0.5023	**2.3469**	0.0902
10 m no spray buffer + 50% drift reduction nozzles						
Ditch	1.13435	0.0019	0.1093	0.2093	0.9779	0.0376
Pond	0.1674	0.0003	0.0161	0.0309	0.1443	0.0055
Stream*	1.3612	0.0022	0.1311	0.2511	**1.1734**	0.0451
10 m no spray buffer + 75% drift reduction nozzles						
Ditch	0.567175	0.0009	0.0546	0.1046	0.4889	0.0188
Pond	0.0837	0.0001	0.0081	0.0154	0.0722	0.0028
Stream*	0.6806	0.0011	0.0656	0.1256	0.5867	0.0225
10 m no spray buffer + 90% drift reduction nozzles						
Ditch	0.22687	0.0004	0.02186	0.04186	0.19558	0.00751
Pond	0.03348	5.47E-05	0.00323	0.00618	0.02886	0.00111
Stream*	0.27224	0.0004	0.02623	0.05023	0.23469	0.00902
20 m no spray buffer						
Ditch	1.1788	0.0019	0.11356	0.21749	**1.01621**	0.03905
Pond	0.2235	0.0004	0.02153	0.04124	0.19267	0.00740
Stream*	1.4146	0.0023	0.13628	0.26100	**1.21948**	0.04686
20 m no spray buffer + 50% drift reduction nozzles						
Ditch	0.5894	0.0010	0.0568	0.1087	0.5081	0.0195
Pond	0.11175	0.0002	0.0108	0.0206	0.0963	0.0037
Stream*	0.7073	0.0012	0.0681	0.1305	0.6097	0.0234
20 m no spray buffer + 75% drift reduction nozzles						
Ditch	0.2947	0.0005	0.0284	0.0544	0.2541	0.0098
Pond	0.055875	9.13E-05	0.0054	0.0103	0.0482	0.0019
Stream*	0.35365	0.0006	0.0341	0.0652	0.3049	0.0117
20 m no spray buffer + 90% drift reduction nozzles						
Ditch	0.11788	0.0002	0.0114	0.0217	0.1016	0.0039
Pond	0.02235	3.65E-05	0.0022	0.0041	0.0193	0.0007
Stream*	0.14146	0.0002	0.0136	0.0261	0.1219	0.0047

PEC/RAC ratios above the relevant trigger of 1 are shown in bold

Formulation GLOB1310aH – Stepwise approach (AGD)

The first step is to check if measured data on the product exist for the given endpoint (**Step 1**). If yes, comparison between product data and active ingredient data will be possible. For GLOB1310aH, we have data on the formulation for *Daphnia*, two species of algae (*P. subcapitata* and *Desmodesmus subspicatus*, most sensitive species for flufenacet and Aclonifen, respectively), and two species of aquatic macrophytes (*Lemna* and *Myriophyllumi)* because the product is an herbicide.

As there is no active ingredient data on *Myriophyllum*, the comparison for this organism is not possible, and therefore *Myriophylum* will be addressed separately using the data obtained in the test with the formulation. In the case of algae, the agreed endpoint for aclonifen is set for *Desmodesmus subspicatus* (most sensitive species for Aclonifen); while, for flufenacet the algal species whit an agreed endpoint is *P. subcapitata* (*P. subcapitata* was confirmed as the most sensitive algal species in the RAR flufenacet). As the endpoint for algae is established in two different algal species (with different sensitivity) for aclonifen and flufenacet, a comparison is not suitable and the risk assessment will be addressed separately using the endpoint of the formulation tests.
The risk assessment is presented below for each aquatic organism group.

Fish

Since the endpoint for fish is only available for the active substances, and the mixture toxicity calculation is feasible (Step 7 AGD), then according to the step approach in the AGD indicates to calculate the mixture toxicity (Step 8). In this case since the AF used in the risk assessment for both active ingredients is the same then a ETRmix is calculated.

Equation 18: $$ETR_{mix-CA} = \frac{PEC_{mix}}{ECx_{mix-CA}}$$

The following results were obtained for Step 4 TOXSWA 10m vegetated buffer strip + 10m no spray buffer (from the AGD_Aquamix_v1.15). For the calculations of the risk assessment to fish the risk envelope approach is applied and the PECsw values were taken from the simulations of 2L GLOB1310aH/ha (1080g Aclonifen/ha + 120 g Flufenacet/ha) at both pre-emergence and post-emergence; as this is the highest requested dose all the other intended doses are covered in this risk assessment.

Fish (2.0L/ha pre-emergence)		
FOCUS Step 4	**Scenario**	**ETRmix-CA**
	Vegetative strip [m]	**10**
Nozzle reduction	**No spray buffer [m]**	**10**
None	D1 Ditch	0.0033
50%		0.0026
75%		0.0022
90%		0.0020
None	D1 Stream	0.0029
50%		0.0020
75%		0.0016
90%		0.0013
None	D2 Ditch	0.0055
50%		0.0047
75%		0.0043

Fish (2.0L/ha post-emergence)		
FOCUS Step 4	**Scenario**	**ETRmix-CA**
	Vegetative strip [m]	**10**
Nozzle reduction	**No spray buffer [m]**	**10**
None	D1 Ditch	0.0035
50%		0.0027
75%		0.0024
90%		0.0021
None	D1 Stream	0.0030
50%		0.0021
75%		0.0017
90%		0.0014
None	D2 Ditch	0.0061
50%		0.0054
75%		0.0050

90%		0.0041
None	D2 Stream	0.0043
50%		0.0034
75%		0.0030
90%		0.0027
None	D3 Ditch	0.0015
50%		0.0008
75%		0.0004
90%		0.0001
None	D4 Pond	0.0004
50%		0.0003
75%		0.0002
90%		0.0002
None	D4 Stream	0.0019
50%		0.0011
75%		0.0006
90%		0.0004
None	D5 Pond	0.0005
50%		0.0004
75%		0.0004
90%		0.0003
None	D5 Stream	0.0022
50%		0.0013
75%		0.0009
90%		0.0006
None	D6 Ditch	0.0030
50%		0.0023
75%		0.0020
90%		0.0020
None	R1 Pond	0.0003
50%		0.0002
75%		0.0002
90%		0.0002
None	R1 Stream	0.0016
50%		0.0015
75%		0.0015
90%		0.0015
None	R2 Stream	-
50%		-
75%		-
90%		-
None	R3 Stream	0.0018
50%		0.0018
75%		0.0018
90%		0.0018
None	R4 Stream	0.0018
50%		0.0018
75%		0.0018
90%		0.0018

90%		0.0048
None	D2 Stream	0.0045
50%		0.0037
75%		0.0033
90%		0.0031
None	D3 Ditch	0.0015
50%		0.0008
75%		0.0004
90%		0.0001
None	D4 Pond	0.0005
50%		0.0004
75%		0.0003
90%		0.0003
None	D4 Stream	0.0020
50%		0.0012
75%		0.0008
90%		0.0005
None	D5 Pond	0.0021
50%		0.0012
75%		0.0008
90%		0.0005
None	D5 Stream	0.0006
50%		0.0005
75%		0.0005
90%		0.0004
None	D6 Ditch	0.0030
50%		0.0023
75%		0.0020
90%		0.0020
None	R1 Pond	0.0003
50%		0.0002
75%		0.0002
90%		0.0002
None	R1 Stream	0.0016
50%		0.0015
75%		0.0015
90%		0.0015
None	R2 Stream	-
50%		-
75%		-
90%		-
None	R3 Stream	0.0022
50%		0.0018
75%		0.0018
90%		0.0018
None	R4 Stream	0.0020
50%		0.0020
75%		0.0020
90%		0.0020

The combined risk to fish is acceptable when a 10m vegetated buffer strip + 10m no spray buffer is respected.

Daphnia

- **Concentration addition model (MDR) (step 2 AGD)**

The LD_{50} of the formulated product is compared to the predicted mixture toxicity assuming concentration additivity according to the concentration addition model (CA model). The CA model is based on the following equation[1], for deriving a predicted ECx or NOEC value for a mixture of (active) substances with known toxicity (ECx_{mix-CA} or $NOEC_{mix-CA}$), assuming concentration additivity:

Equation 13: $$ECx_{mix-CA} = \left(\sum_{i=1}^{n} \frac{p_i}{ECx_i} \right)^{-1}$$

where:

n: number of mixture components
i: index from 1…n mixture components
p_i: the i^{th} component as a relative fraction of the mixture composition (note: $\Sigma\, p_i$ must be 1)
ECx_i: concentration of component i provoking x % effect (pragmatically, $NOEC_i$ may be inserted, too).

When the formulation is more toxic than that predicted from the toxicity of the individual compounds, the use of the endpoint of the formulation is recommended for the first-tier assessment because it cannot be excluded that such effects would also occur after exposure of the aquatic organism to residues in the environment.

Table 0-24: Comparison of the toxicity of GLOB1310aH to the predicted one based on the active ingredients

Aquatic organisms	Fraction of Aclonifen in mixture	Fraction of Flufenacet in mixture	Aclonifen EC_{50} (mg a.s./L)	Flufenacet EC_{50} (mg a.s./L)	$ECx_{mix-CA.}$ Predicted EC_{50} of GLOB1310aH based on the a.s. toxicity (mg a.s./L)	$ECx_{ppp.}$ EC_{50} of GLOB1310aH from the studies (mg sum of a.s./L)	MDR (model deviation ratio)	Comparison toxicity of the formulation and the predicted one
Daphnia	0.9	0.1	1.2	30.9	1.328	3.017	0.44	MDR= 0.2-5

The predicted toxicity endpoint has been compared to the formulated product endpoint to derive a MDR by the formula ($MDR = ECx_{mix-CA} / ECx_{ppp}$). If MDR is between 0.2 and 5, the observed and calculated toxicities are considered in agreement. If MDR is > 5, the observed toxicity of mixture is higher than that calculated assuming dose additivity. If MDR is < 0.2, the mixture is less toxic than expected.

The MDR for Daphnia is between 0.2 and 5, thus the measured and calculated toxicity are in agreement. It means that the toxicity of GLOB1310aH is not higher than the predicted one. In this case, EFSA (2013) recommends that the measured toxicity of the mixture be considered in the aquatic risk assessment (see Step 3 below).

- **Mixture composition in the formulation versus mixture composition at PECmix (step 3 AGD)**

The aim of this step is to check whether the mixture composition in the formulation study giving the measured mixture toxicity (ECxPPP) in terms of the relative proportions of the individual active substances is similar to the mixture composition at the PECmix (proportion of each active in the environment (part of the PEC)).

The same equation (equation 13) as for step 2 is used, with the difference that here the pi is PECi/PECmix. PECmix is simply the sum the each PECi.

Using the same Ecxmix-CA (a.s. in PPP) as the one used in step 2 for MDR, the new Ecxmix-CA (a.s. in PECmix) (representing the mixture as it is in the environment) is calculated.

The following results were obtained for Step 1, 2 and 3 TOXSWA (from the AGD_Aquamix_v1.15). For the calculations of the risk assessment to Daphnia the risk envelope approach is applied and the PECsw values were taken from the simulations of 2L GLOB1310aH/ha (1080g Aclonifen/ha + 120 g Flufenacet/ha) as this is the highest requested dose all the other intended doses are covered in this risk assessment.

Invertebrates (2L formulation/ha, pre-emergence)	
ECxmix-CA (a.s. in PPP)/ ECxmix-CA (a.s. in PECmix)	
Step 1	0.70
Step 2	
N-Europe	0.70
S-Europe	0.70
Step 3	
D1 Ditch	0.72
D1 Stream	0.80
D2 Ditch	0.52
D2 Stream	0.61
D3 Ditch	1.00
D4 Pond	0.46
D4 Stream	1.00
D5 Pond	0.33
D5 Stream	0.98
D6 Ditch	0.76
R1 Pond	0.96
R1 Stream	0.67
R2 Stream	
R3 Stream	0.68
R4 Stream	0.90

In D3 ditch, D4 stream, D5 stream, R1 Pond, R4 stream the Ecxmix-CA (a.s. in PPP)/Ecxmix-CA (a.s. in PECmix) is within 0.8–1.2 (mixture similar), thus further evaluation is needed at Step 4. In most of the cases the Ecxmix-CA (a.s. in PPP)/Ecxmix-CA (a.s. in PECmix) is not within 0.8–1.2, thus the mixture is not similar (except in D3 ditch, D4 stream, D5 stream, R1 Pond, R4 stream) and further evaluation at Step 5 is done.

- **Measured risk assessment- ETRmix-PPP based on product data (step 4 AGD)**

In this step, the ETRmix-PPP is calculated, i.e. the ratio between the PECmix and the measured mixture toxicity (i.e. endpoint from studies conducted with the PPP and expresses as EcxPP (mg sum a.s./L). The ETRmix-PPP is then compared with the ETR-trigger values.

Final CONCLUSION	low risk
	high risk

Invertebrates (2L formulation/ha pre-emergence)	
ETRmix-PPP	
Step 1	Go to 5/8
Step 2	
N-Europe	Go to 5/8
S-Europe	Go to 5/8
Step 3	
D1 Ditch	Go to 5/8
D1 Stream	Go to 5/8
D2 Ditch	Go to 5/8
D2 Stream	Go to 5/8
D3 Ditch	0.00
D4 Pond	Go to 5/8
D4 Stream	0.00
D5 Pond	Go to 5/8
D5 Stream	0.00
D6 Ditch	Go to 5/8
R1 Pond	0.00
R1 Stream	Go to 5/8
R2 Stream	
R3 Stream	Go to 5/8
R4 Stream	0.00

The combined risk for Daphnia from the use of GLOB1310aH (2L/ha pre-emergence) is acceptable in the scenarios D3 ditch, D4 stream, D5 stream, R1 Pond, R4 stream for the use at pre-emergence. For the other scenarios, further assessment is necessary at Step 5 AGD.

The combined risk for Daphnia from the use of GLOB1310aH (2L/ha post-emergence) is acceptable in the scenarios D3 ditch, D4 stream, D5 pond, R1 Pond for the use at post-emergence. For the other scenarios, further assessment is necessary at Step 5 AGD.

- **Identification of toxicity driver (step 5 AGD)**

Following the EFSA Aquatic guidance document[1], the check of a single drive for the toxicity was made according to the following formula:

$$\sum_{i=1}^{n} TU_i = \sum_{i=1}^{n} \frac{c_i}{ECx_i}$$

in which TU is the ratio between the concentration (i.e. ci) of a mixture component and its toxicological acute (e.g. EC50) or chronic (e.g. long-term NOEC) endpoint.

The other calculations are:

Toxicity of the sum of active ingredients (TOXsum(ai)) = 1/(TU(ai1) + TU(ai2))
Contribution to toxicity = TOXsum(ai) * TU(ai) * 100

Table 0-25: Identification of the toxicity driver for Daphnia

Organism	Active substance	EC_{50} (mg/L)	Fraction in mixture	Toxic unit	Tox of the sum ai	Contribution to toxicity
Daphnia	Aclonifen	1.2	0.9	0.7500	1.328	99.57
	Flufenacet	30.9	0.1	0.0032		0.43

For Daphnia the toxicity is clearly driven by Aclonifen (contribution ≥90%). Therefore, in accordance with the EFSA guidance document the risk assessment for Daphnia can be based on single-substance toxicity data (Ecxa.s.) for the identified 'driver' of mixture toxicity, which is in this case aclonifen. Therefore, reference is made to the risk assessment performed with aclonifen.

Lemna

- **Concentration addition model (MDR) (step 2 AGD)**

The LD_{50} of the formulated product is compared to the predicted mixture toxicity assuming concentration additivity according to the concentration addition model (CA model). The CA model is based on the following equation[1], for deriving a predicted ECx or NOEC value for a mixture of (active) substances with known toxicity ($ECx_{mix\text{-}CA}$ or $NOEC_{mix\text{-}CA}$), assuming concentration additivity:

Table 0-26: Comparison of the toxicity of GLOB1310aH to the predicted one based on the active ingredients

Aquatic organisms	Fraction of Aclonifen in mixture	Fraction of Flufenacet in mixture	Aclonifen EC_{50} (mg a.s./L)	Flufenacet EC_{50} (mg a.s./L)	$ECx_{mix\text{-}CA}$. Predicted EC_{50} of GLOB1310aH based on the a.s. toxicity (mg a.s./L)	ECx_{ppp}. EC_{50} of GLOB1310aH from the studies (mg sum of a.s./L)	MDR (model deviation ratio)	Comparison toxicity of the formulation and the predicted one
Lemna	0.9	0.1	0.012	0.00243	0.009	0.0057	1.5	MDR= 0.2-5

The predicted toxicity endpoint has been compared to the formulated product endpoint to derive a MDR by the formula (MDR = $Ecx_{mix\text{-}CA}$ /ECx_{ppp}). If MDR is between 0.2 and 5, the observed and calculated toxicities are considered in agreement. If MDR is > 5, the observed toxicity of mixture is higher than that calculated assuming dose additivity. If MDR is < 0.2, the mixture is less toxic than expected.

The MDR for Lemna is between 0.2 and 5, thus the measured and calculated toxicity are in agreement. No antagonism or synergism is indicated. It means that the toxicity of GLOB1310aH is not higher than the predicted one. Since the observed and calculated toxicities are considered in agreement, the measured mixture toxicity can be used for the risk assessment. Taking into account that different assessment factors (for flufenacet a microcosm study with AF of 5 is used as refined risk assessment) and additional data are available, a refined risk assessment using the Rqmix (Step 8b) is performed, according to the EFSA Aquatic guidance document.

- **Risk assessment (refined) using Rqmix (Step 8b AGD)**

In this step, a formula yielding a risk quotient for the mixture (Rqmix) is used to evaluate the risk (according to equation 21). In this formula the ratio between the PECi (PEC of the individual substances), and the RACi (RAC of individual substances are summarised. The Rqmix >1 indicates an acceptable risk. The calculation of the mixture toxicity is based on the regulatory acceptable concentration of the individual a.s. (RAC*i*) using the following formula yielding a risk quotient for the mixture:

Equation 21:

$$RQ_{mix} = \sum_{i=1}^{n} \frac{PEC_i}{RAC_i}$$

For aclonifen the endpoint from Tier I study in *Lemna* (12 µg/L) in combination with the assessment factor of 10 is used, leading to a RAC of 1.2 µg/L. For flufenacet, the endpoint from the microcosms study in combination with an assessment factor of 5 is used, leading to a RAC of 2.4µg/L.

A restriction for not using the product on artificial drained soil (D1, D2 and D6) is already foreseen due to flufenacet, these scenarios are therefore not considered in these combined risk assessments.

The Rqmix was calculated considering the Step 4 calculation obtained with the FOCUS Landscape and Mitigation Report and those obtained with VFSMOD are presented separately.

Table 0-27: Rqmix of GLOB1310aH for *Lemna* – Use 1 1.5L/ha pre-emergence (Step 4)

75% drift reduction nozzles			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	1.058	0.072	**1.1**
D4 (pond)	0.055	0.114	0.2
D4 (stream)	1.236	0.136	**1.4**
D5 (pond)	0.055	0.199	0.3
D5 (stream)	1.333	0.264	**1.6**
R1 (pond)	0.186	0.013	0.2
R1 (stream)	1.028	0.954	**2.0**
R3 (stream)	1.304	1.271	**2.6**
R4 (stream)	1.523	0.344	**1.9**
90% drift reduction nozzles			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	0.422	0.029	0.5
D4 (pond)	0.022	0.113	0.1
D4 (stream)	0.494	0.136	0.6
D5 (pond)	0.022	0.198	0.2
D5 (stream)	0.533	0.264	0.8
R1 (pond)	0.175	0.012	0.2
R1 (stream)	1.028	0.954	**2.0**
R3 (stream)	1.173	1.271	**2.4**
R4 (stream)	1.523	0.344	**1.9**
5m no spray buffer			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	1.147	0.064	**1.2**
D4 (pond)	0.126	0.115	0.2
D4 (stream)	1.340	0.136	**1.5**
D5 (pond)	0.126	0.200	0.3
D5 (stream)	1.446	0.264	**1.7**
R1 (pond)	0.211	0.016	0.2
R1 (stream)	1.028	0.954	**2.0**
R3 (stream)	1.414	1.271	**2.7**
R4 (stream)	1.523	0.344	**1.9**

5m no spray buffer + 50% drift reduction nozzles			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	0.573	0.032	0.6
D4 (pond)	0.063	0.114	0.2
D4 (stream)	0.669	0.136	0.8
D5 (pond)	0.063	0.199	0.3
D5 (stream)	0.722	0.264	1.0
R1 (pond)	0.189	0.013	0.2
R1 (stream)	1.028	0.954	**2.0**
R3 (stream)	1.173	1.271	**2.4**
R4 (stream)	1.523	0.344	**1.9**
10m no spray buffer			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	0.608	0.034	0.6
D4 (pond)	0.091	0.114	0.2
D4 (stream)	0.710	0.136	0.8
D5 (pond)	0.091	0.199	0.3
D5 (stream)	0.766	0.264	1.0
R1 (pond)	0.199	0.014	0.2
R1 (stream)	1.028	0.954	**2.0**
R3 (stream)	1.173	1.271	**2.4**
R4 (stream)	1.523	0.344	**1.9**
10 m vegetated strip +10 m no spray buffer			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	0.607	0.034	0.6
D4 (pond)	0.091	0.114	0.2
D4 (stream)	0.710	0.136	0.8
D5 (pond)	0.091	0.199	0.3
D5 (stream)	0.766	0.264	1.0
R1 (pond)	0.100	0.008	0.1
R1 (stream)	0.540	0.428	1.0
R3 (stream)	0.757	0.580	**1.3**
R4 (stream)	0.794	0.155	0.9
10 m vegetated strip +10 m no spray buffer + 50% drift reduction nozzles			
D3 (ditch)	0.304	0.017	0.3
D4 (pond)	0.045	0.113	0.2
D4 (stream)	0.355	0.136	0.5
D5 (pond)	0.045	0.199	0.2
D5 (stream)	0.383	0.264	0.6
R1 (pond)	0.083	0.006	0.1
R1 (stream)	0.460	0.428	0.9
R3 (stream)	0.535	0.580	1.1
R4 (stream)	0.687	0.155	0.8
20m vegetated strip + 20m no spray buffer			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	0.315	0.315	0.3
D4 (pond)	0.061	0.061	0.1
D4 (stream)	0.369	0.369	0.4
D5 (pond)	0.061	0.061	0.1
D5 (stream)	0.398	0.398	0.4
R1 (pond)	0.061	0.061	0.1
R1 (stream)	0.280	0.280	0.3
R3 (stream)	0.389	0.389	0.4
R4 (stream)	0.358	0.358	0.4

In the table above it is observed that the Rqmix for Lemna in the R3 stream scenario is slightly high than 1, namely, 1.1, when a 10m vegetated buffer strip + 10m no spray buffer or 10m vegetated filter strip + 50 m no spray buffer. However, it has to be highlighted that the AF of 5 used for the PEC/RAC calculations of flufenacet, as explained in point 9.5.1.1, is very conservative. As indicated in the EFSA Guidance for aquatic risk assessment an AF of 2 should be normally applied. Thus this Rqmix of 1.1 is acceptable and can be considered protective for Lemna, thus 20m vegetated filter strip + 20 m no psray buffer is not necessary.

For the Use 1, the combined risk to *Lemna* is acceptable when one of the following measures are followed:

- D scenarios:
    - 90% drift reduction nozzles OR
    - 5m no spray buffer + 50% drift reduction nozzles OR
    - 10m no spray buffer
- Run-off susceptible soils:
    - 10m vegetated strip + 10m no spray buffer + 50% drift reduction nozzles OR

For the Member states that considered Step 4 calculation with VFSMod acceptable, the combined risk assessment for Use 1 is presented below. The PEC/RAC values of aclonifen were obtained using the PECsw values obtained with VFSMod, while the PEC/RAC ratios of flufenacet ware those obtained with FOCUS Landscape Mitigation Report.

Table 0-28: Rqmix of GLOB1310aH for *Lemna* – Use 1 1.5L/ha pre-emergence (Step 4 – VFSMod)

10 m vegetated strip +10 m no spray buffer			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
R1 (pond)	0.091	0.008	0.1
R1 (steam)	0.540	0.428	1.0
R3 (stream)	0.749	0.580	**1.3**
R4 (stream)	0.543	0.155	0.7
10 m vegetated strip +10 m no spray buffer + 50% drift reduction nozzles			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
R1 (pond)	0.045	0.006	0.1
R1 (steam)	0.270	0.428	0.7
R3 (stream)	0.432	0.580	1.0
R4 (stream)	0.271	0.155	0.4

Based on the PECsw obtained with VFSMod for the Use 1, the combined risk to *Lemna* is acceptable when the following restriction is applied in run-off susceptible soils:

- 10m vegetated strip + 10m no spray buffer + 50% drift reduction nozzles

Table 0-29: Rqmix of GLOB1310aH for Lemna – Use 2 2.0L/ha pre-emergence (Step 4)

75% drift reduction nozzles			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	1.410	0.096	**1.5**
D4 (pond)	0.073	0.152	0.2
D4 (stream)	1.648	0.185	**1.8**
D5 (pond)	0.073	0.265	0.3

D5 (stream)	1.778	0.355	**2.1**
R1 (pond)	0.256	0.018	0.3
R1 (stream)	1.421	1.297	**2.7**
R3 (stream)	1.739	1.731	**3.5**
R4 (stream)	2.103	0.453	**2.6**
90% drift reduction nozzles			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	0.564	0.038	0.6
D4 (pond)	0.029	0.151	0.2
D4 (stream)	0.659	0.185	0.8
D5 (pond)	0.029	0.264	0.3
D5 (stream)	0.711	0.355	**1.1**
R1 (pond)	0.241	0.016	0.3
R1 (stream)	1.421	1.297	**2.7**
R3 (stream)	1.626	1.731	**3.4**
R4 (stream)	2.103	0.453	**2.6**
5m no spray buffer			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	1.529	0.086	**1.6**
D4 (pond)	0.168	0.153	0.3
D4 (stream)	1.787	0.185	**2.0**
D5 (pond)	0.168	0.266	0.4
D5 (stream)	1.928	0.355	**2.3**
R1 (pond)	0.289	0.022	0.3
R1 (stream)	1.421	1.297	**2.7**
R3 (stream)	1.886	1.731	**3.6**
R4 (stream)	2.103	0.453	**2.6**
5m no spray buffer + 50% drift reduction nozzles			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	0.764	0.043	0.8
D4 (pond)	0.084	0.145	0.2
D4 (stream)	0.893	0.185	**1.1**
D5 (pond)	0.084	0.265	0.3
D5 (stream)	0.963	0.355	**1.3**
R1 (pond)	0.260	0.018	0.3
R1 (stream)	1.421	1.297	**2.7**
R3 (stream)	1.626	1.731	**3.4**
R4 (stream)	2.103	0.453	**2.6**
5m no spray buffer + 75% drift reduction nozzles			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	0.382	0.021	0.4
D4 (pond)	0.042	0.151	0.2
D4 (stream)	0.446	0.185	0.6
D5 (pond)	0.042	0.264	0.3
D5 (stream)	0.481	0.355	0.8
R1 (pond)	0.245	0.016	0.3
R1 (stream)	1.421	1.297	**2.7**
R3 (stream)	1.626	1.731	**3.4**
R4 (stream)	2.103	0.453	**2.6**
10 m no spray buffer			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	0.810	0.045	0.9
D4 (pond)	0.121	0.152	0.3
D4 (stream)	0.947	0.185	**1.1**
D5 (pond)	0.121	0.266	0.4
D5 (stream)	1.022	0.355	**1.4**
R1 (pond)	0.273	0.020	0.3
R1 (stream)	1.421	1.297	**2.7**

R3 (stream)	1.626	1.731	**3.4**
R4 (stream)	2.103	0.453	**2.6**
10m no spray buffer + 50% drift reduction nozzles			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	0.405	0.227	0.6
D4 (pond)	0.060	0.152	0.2
D4 (stream)	0.473	0.185	0.7
D5 (pond)	0.060	0.265	0.3
D5 (stream)	0.510	0.355	0.9
R1 (pond)	0.251	0.017	0.3
R1 (stream)	1.421	1.297	**2.7**
R3 (stream)	1.626	1.731	**3.4**
R4 (stream)	2.103	0.453	**2.6**
10 m vegetated strip +10 m no spray buffer			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	0.810	0.045	0.856
D4 (pond)	0.121	0.152	0.273
D4 (stream)	0.947	0.185	**1.131**
D5 (pond)	0.121	0.266	0.387
D5 (stream)	1.022	0.355	**1.376**
R1 (pond)	0.136	0.011	0.1
R1 (stream)	0.720	0.581	**1.3**
R3 (stream)	0.999	0.790	**1.8**
R4 (stream)	0.948	0.205	**1.2**
10 m vegetated strip +10 m no spray buffer + 50% drift reduction nozzles			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	0.405	0.023	0.42
D4 (pond)	0.060	0.152	0.21
D4 (stream)	0.473	0.185	0.65
D5 (pond)	0.060	0.265	0.32
D5 (stream)	0.510	0.355	0.86
R1 (pond)	0.114	0.008	0.1
R1 (steam)	0.635	0.581	**1.2**
R3 (stream)	0.741	0.790	**1.5**
R4 (stream)	0.948	0.205	**1.1**
10 m vegetated strip +10 m no spray buffer + 90% drift reduction nozzles			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	0.081	0.005	0.08
D4 (pond)	0.012	0.151	0.16
D4 (stream)	0.094	0.185	0.27
D5 (pond)	0.012	0.264	0.27
D5 (stream)	0.102	0.355	0.45
R1 (pond)	0.097	0.006	0.1
R1 (stream)	0.635	0.581	**1.2**
R3 (stream)	0.741	0.790	**1.5**
R4 (stream)	0.948	0.205	**1.2**
20 m vegetated strip +20 m no spray buffer			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
D3 (ditch)	0.421	0.024	0.44
D4 (pond)	0.081	0.152	0.23
D4 (stream)	0.492	0.185	0.67
D5 (pond)	0.081	0.265	0.34
D5 (stream)	0.531	0.355	0.88
R1 (pond)	0.081	0.007	0.1
R1 (stream)	0.374	0.303	0.7
R3 (stream)	0.519	0.414	0.9
R4 (stream)	0.495	0.107	0.6

Based on Step 4 (Landscape Mitigation Report), for the Use ~~3~~ 2, the combined risk to Lemna is acceptable when the following restriction is applied:

- D scenarios:
    - 5m no spray buffer + 75% drift reduction nozzles OR
    - 10m no spray buffer + 50% drift reduction nozzles
- Run-off susceptible soils:
    - 20m vegetated strip + 20m no spray buffer

For the Member states that considered Step 4 calculation with VFSMod acceptable the combined risk assessment for Use ~~3~~ 2 is presented below.

Table 0-30: Rqmix of GLOB1310aH for Lemna – Use 2 2.0L/ha pre-emergence (Step 4 VFSMod)

10 m vegetated strip +10 m no spray buffer			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
R1 (pond)	0.121	0.011	0.1
R1 (steam)	0.720	0.581	**1.3**
R3 (stream)	0.999	0.790	**1.8**
R4 (stream)	0.724	0.205	0.9
10 m vegetated strip +10 m no spray buffer + 50% drift reduction nozzles			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
R1 (pond)	0.060	0.008	0.0
R1 (steam)	0.359	0.581	0.9
R3 (stream)	0.599	0.790	**1.3**
R4 (stream)	0.362	0.205	0.5
10 m vegetated strip +10 m no spray buffer + 75% drift reduction nozzles			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
R1 (pond)	0.030	0.007	0.0
R1 (stream)	0.180	0.581	0.8
R3 (stream)	0.599	0.790	**1.3**
R4 (stream)	0.181	0.205	0.4
20 m vegetated strip +20 m no spray buffer			
Scenario	**PEC/RAC Aclonifen**	**PEC/RAC Flufenacet**	**Rqmix**
R1 (pond)	0.081	0.007	0.1
R1 (stream)	0.374	0.303	0.7
R3 (stream)	0.519	0.414	0.9
R4 (stream)	0.376	0.107	0.5

Based on Step 4 (VFSMod), for the Use ~~3~~ 2, the combined risk to *Lemna* is acceptable when the following is applied:

- Run-off susceptible soils:
    - 20m vegetated strip + 20m no spray buffer

Alga - *Pseudokirchneriella subcapitata*

For *P. subcapitata*, no data is available for the active substance aclonifen, but only for flufenacet and thus the comparison with aclonifen cannot be made. The risk assessment for *P. subcapitata* will be performed with the product GLOB1310aH in absence of any other supportive data (no aclonifen endpoint of P.

sucapitata is available). The RAC of 2.66 µg/L, (ErC50 of 26.62µg active substance/L, combined with an AF of 10) based on measured mixture toxicity, is compared to the PECmix, which is calculated as the sum of PECsw of the individual active substances (obtained at Step4 calculation at which the risk of the active ingredients individually was solved). The individual PECsw obtained at Step 4, the PECmix and the PEC/RAC ratio can be found in the table below.

A restriction for not using the product on artificial drained soil (D1, D2 and D6) is already foreseen due to flufenacet, these scenarios are therefore not considered in these combined risk assessments.

Table 0-31: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for GLOB1310aH for *P. subcapitata* based on PECmix calculations for Use 1 (1.5L/ha pre-emergence)

Scenario	PECsw Aclonifen (µg/L)	PECsw Flufenacet (µg/L)	PECsw mix (µg a.s./L)	RAC 2.66 µg/L
	50% drift reduction nozzles			
D3 (ditch)	2.540	0.346	2.886	**1.1**
D4 (pond)	0.131	0.275	0.406	0.2
D4 (stream)	2.967	0.404	3.371	**1.3**
D5 (pond)	0.131	0.479	0.610	0.2
D5 (stream)	3.202	0.634	3.836	**1.4**
R1 (pond)	0.246	0.038	0.284	0.1
R1 (stream)	2.255	2.289	4.544	**1.7**
R3 (stream)	3.132	3.051	6.183	**2.3**
R4 (stream)	2.269	0.827	3.096	**1.2**
Scenario	**PECsw Aclonifen (µg/L)**	**PECsw Flufenacet (µg/L)**	**PECsw mix (µg a.s./L)**	**RAC 2.66 µg/L**
	75% drift reduction nozzles			
D3 (ditch)	1.269	0.173	1.442	0.5
D4 (pond)	0.066	0.273	0.338	0.1
D4 (stream)	1.483	0.327	1.810	0.7
D5 (pond)	0.066	0.477	0.542	0.2
D5 (stream)	1.600	0.634	2.234	0.8
R1 (pond)	0.223	0.031	0.255	0.1
R1 (stream)	1.234	2.289	3.523	**1.3**
R3 (stream)	1.565	3.051	4.616	**1.7**
R4 (stream)	1.827	0.827	2.654	**1.0**
Scenario	**PECsw Aclonifen (µg/L)**	**PECsw Flufenacet (µg/L)**	**PECsw mix (µg a.s./L)**	**RAC 2.66 µg/L**
	5m no spray buffer			
D3 (ditch)	1.376	0.154	1.530	0.6
D4 (pond)	0.152	0.275	0.426	0.2
D4 (stream)	1.608	0.327	1.935	0.7
D5 (pond)	0.152	0.479	0.631	0.2
D5 (stream)	1.735	0.634	2.369	0.9
R1 (pond)	0.253	0.039	0.292	0.1
R1 (stream)	1.234	2.289	3.523	**1.3**
R3 (stream)	1.697	3.051	4.748	**1.8**
R4 (stream)	1.827	0.827	2.654	**1.0**
Scenario	**PECsw Aclonifen (µg/L)**	**PECsw Flufenacet (µg/L)**	**PECsw mix (µg a.s./L)**	**RAC 2.66 µg/L**

	10 m vegetated buffer strip + 10m no spray buffer			
D3 (ditch)	0.728	0.082	0.810	0.305
D4 (pond)	0.109	0.274	0.383	0.144
D4 (stream)	0.852	0.327	1.179	0.443
D5 (pond)	0.109	0.478	0.587	0.221
D5 (stream)	0.919	0.634	1.554	0.584
R1 (pond)	0.120	0.020	0.140	0.053
R1 (stream)	0.647	1.026	1.673	0.629
R3 (stream)	0.909	1.392	2.301	0.865
R4 (stream)	0.952	0.373	1.326	0.498

Based on PECsw Step 4 for the Use 1, the combined risk to *P. subcapitata* is acceptable when the following is applied:

- D scenarios:
  - 75% drift reduction nozzles OR
  - 5m no spray buffer
- Run-off susceptible scenarios:
  - 10 m vegetated buffer strip + 10m no spray buffer

Table 0-32: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for GLOB1310aH for *P. subcapitata* based on PECmix calculations for Use 2 (2L/ha pre-emergence)

Scenario	**PECsw Aclonifen (µg/L)**	**PECsw Flufenacet (µg/L)**	**PECsw mix (µg a.s./L)**	**RAC 2.66 µg/L**
	75% drift reduction nozzles			
D3 (ditch)	1.692	0.231	1.923	0.7
D4 (pond)	0.087	0.365	0.452	0.2
D4 (stream)	1.977	0.443	2.420	0.9
D5 (pond)	0.087	0.636	0.723	0.3
D5 (stream)	2.134	0.852	2.986	**1.1**
R1 (pond)	0.307	0.042	0.349	0.1
R1 (stream)	1.705	3.113	4.818	**1.8**
R3 (stream)	2.087	4.155	6.242	**2.3**
R4 (stream)	2.523	1.088	3.611	**1.4**
Scenario	**PECsw Aclonifen (µg/L)**	**PECsw Flufenacet (µg/L)**	**PECsw mix (µg a.s./L)**	**RAC 2.66 µg/L**
	90% drift reduction nozzles			
D3 (ditch)	0.676	0.092	0.769	0.3
D4 (pond)	0.035	0.363	0.398	0.1
D4 (stream)	0.790	0.443	1.233	0.5
D5 (pond)	0.035	0.634	0.669	0.3
D5 (stream)	0.853	0.852	1.704	0.6
R1 (pond)	0.289	0.037	0.326	0.1
R1 (stream)	1.705	3.113	4.818	**1.8**
R3 (stream)	1.951	4.155	6.106	**2.3**
R4 (stream)	2.523	1.088	3.611	**1.4**
Scenario	**PECsw Aclonifen (µg/L)**	**PECsw Flufenacet (µg/L)**	**PECsw mix (µg a.s./L)**	**RAC 2.66 µg/L**
	5m no spray buffer			
D3 (ditch)	1.835	0.206	2.041	0.8

D4 (pond)	0.202	0.368	0.570	0.2
D4 (stream)	2.144	0.443	2.587	1.0
D5 (pond)	0.202	0.639	0.841	0.3
D5 (stream)	2.314	0.852	3.166	**1.2**
R1 (pond)	1.705	3.113	4.818	**1.8**
R1 (stream)	2.263	4.155	6.418	**2.4**
R3 (stream)	2.523	1.088	3.611	**1.4**
R4 (stream)	2.523	1.088	3.611	**1.4**
Scenario	**PECsw Aclonifen (µg/L)**	**PECsw Flufenacet (µg/L)**	**PECsw mix (µg a.s./L)**	**RAC 2.66 µg/L**
	5m no spray buffer + 50% drift reduction nozzles			
D3 (ditch)	0.917	0.103	1.020	0.4
D4 (pond)	0.101	0.347	0.448	0.2
D4 (stream)	1.071	0.443	1.514	0.6
D5 (pond)	0.101	0.636	0.737	0.3
D5 (stream)	1.156	0.852	2.008	0.8
R1 (pond)	0.311	0.043	0.354	0.1
R1 (stream)	1.705	3.113	4.818	**1.8**
R3 (stream)	1.951	4.155	6.106	**2.3**
R4 (stream)	2.523	1.088	3.611	**1.4**
Scenario	**PECsw Aclonifen (µg/L)**	**PECsw Flufenacet (µg/L)**	**PECsw mix (µg a.s./L)**	**RAC 2.66 µg/L**
	10m no spray buffer			
D3 (ditch)	0.972	0.109	1.082	0.4
D4 (pond)	0.145	0.366	0.511	0.2
D4 (stream)	1.136	0.443	1.579	0.6
D5 (pond)	0.145	0.637	0.783	0.3
D5 (stream)	1.226	0.852	2.078	0.8
R1 (pond)	0.327	0.047	0.374	0.1
R1 (stream)	1.705	3.113	4.818	**1.8**
R3 (stream)	1.951	4.155	6.106	**2.3**
R4 (stream)	2.523	1.088	3.611	**1.4**
Scenario	**PECsw Aclonifen (µg/L)**	**PECsw Flufenacet (µg/L)**	**PECsw mix (µg a.s./L)**	**RAC 2.66 µg/L**
	10 m vegetated buffer strip + 10m no spray buffer			
D3 (ditch)	0.972	0.109	1.082	0.407
D4 (pond)	0.145	0.366	0.511	0.192
D4 (stream)	1.136	0.443	1.579	0.594
D5 (pond)	0.145	0.637	0.783	0.294
D5 (stream)	1.226	0.852	2.078	0.781
R1 (pond)	0.163	0.02695	0.190	0.1
R1 (stream)	0.864	1.395	2.259	0.8
R3 (stream)	1.199	1.896	3.095	**1.2**
R4 (stream)	1.138	0.4911	1.629	0.6
Scenario	**PECsw Aclonifen (µg/L)**	**PECsw Flufenacet (µg/L)**	**PECsw mix (µg a.s./L)**	**RAC 2.66 µg/L**
	10 m vegetated buffer strip + 10m no spray buffer + 50% drn			
D3 (ditch)	0.486	0.05451	0.540	0.2
D4 (pond)	0.073	0.3639	0.436	0.2
D4 (stream)	0.568	0.4432	1.011	0.4
D5 (pond)	0.073	0.6352	0.708	0.3
D5 (stream)	0.612	0.8515	1.464	0.6
R1 (pond)	0.137	0.02015	0.157	0.1

R1 (stream)	0.763	1.395	2.158	0.8
R3 (stream)	0.890	1.896	2.786	1.0
R4 (stream)	1.138	0.4911	1.629	0.6
Scenario	**PECsw Aclonifen (µg/L)**	**PECsw Flufenacet (µg/L)**	**PECsw mix (µg a.s./L)**	**RAC 2.66 µg/L**
	10 m vegetated buffer strip + 50m no spray buffer			
D3 (ditch)	0.2088	0.02346	0.232	0.1
D4 (pond)	0.05043	0.3633	0.414	0.2
D4 (stream)	0.244	0.4432	0.687	0.3
D5 (pond)	0.05044	0.6346	0.685	0.3
D5 (stream)	0.2633	0.8515	1.115	0.4
R1 (pond)	0.129	0.01809	0.147	0.1
R1 (stream)	0.7625	1.395	2.158	0.8
R3 (stream)	0.8895	1.896	2.786	1.0
R4 (stream)	1.138	0.4911	1.629	0.6

Based on PECsw Step 4 for the Use 3, the combined risk to *P. subcapitata* is acceptable when the following is applied:

- D scenarios:
    - 90% drift reduction nozzles OR
    - 5m no spray buffer + 50% drift reduction nozzles OR
    - 10 m no spray buffer
- Run-off susceptible scenarios:
    - 10 m vegetated filter strip + 50 m no spray buffer

Desmodesmus subspicatus

For *Desmodesmus subspicatus* (aclonifen's most sensitive algal species), no data is available for the active substance flufenacet, but only for aclonifen and thus the comparison cannot be made. The risk assessment for *D. subspicatus* will be performed with the product GLOB1310aH in absence of any other supportive data. The RAC of 5.11 µg/L, (ErC50 of 51.1 µg a.s./L, combined with an AF of 10) based on measured mixture toxicity. However, since the RAC value of *Desmodesmus subspicatus* (10.37 µg/L) is more than two times higher than that of *P. subcapitata* (4µg/L) it is considered that the risk assessment to algae (including *D. subspicatus*) is covered by the risk assessment of *P. subcapiata* and no further consideration is necessary for *D. subspicatus.*

Myriophyllum spicatum

For *M. spicatum*, no data is available for the active substances aclonifen and flufenacet. Thus the risk assessment for *M. spicatum* will be performed with the product GLOB1310aH in absence of any other supportive data. The RAC of 14.87 µg/L, (ErC50 of 148.7 µg a.s./L, combined with an AF of 10) based on measured mixture toxicity, is compared to the PECmix, which is calculated as the sum of PECsw of the individual active substances (obtained at Step4 calculation at which the risk of the active ingredients individually was solved). The individual PECsw, the PECmix and the PEC/RAC ratio can be found in the table below.

A restriction for not using the product on artificial drained soil (D1, D2 and D6) is already foreseen due to flufenacet, these scenarios are therefore not considered in these combined risk assessments.

Table 0-33: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for GLOB1310aH for *M. spicatum* based on PECmix calculations for Use 1 (1.5L/ha pre-emergence)

Scenario	PECsw Step 3 Aclonifen (µg/L)	PECsw Step 3 Flufenacet (µg/L)	PECsw mix (µg a.s./L)	RAC 14.87µg a.s./L
D3 (ditch)	5.082	0.569	5.651	0.380
D4 (pond)	0.263	0.276	0.538	0.036
D4 (stream)	5.937	0.493	6.430	0.432
D5 (pond)	0.263	0.480	0.742	0.050
D5 (stream)	6.406	0.634	7.040	0.473
R1 (pond)	0.293	0.041	0.334	0.022
R1 (stream)	4.512	2.289	6.801	0.457
R3 (stream)	6.266	3.051	9.317	0.627
R4 (stream)	4.540	0.827	5.367	0.361

Table 0-34: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for GLOB1310aH for *M. spicatum* based on PECmix calculations for Use 2 (2L/ha pre-emergence)

Scenario	PECsw Step 3 Aclonifen (µg/L)	PECsw Step 3 Flufenacet (µg/L)	PECsw mix (µg a.s./L)	RAC 14.87µg a.s./L
D3 (ditch)	6.780	0.759	7.539	0.507
D4 (pond)	0.350	0.368	0.719	0.048
D4 (stream)	7.921	0.658	8.579	0.577
D5 (pond)	0.350	0.640	0.990	0.067
D5 (stream)	8.546	0.852	9.398	0.632
R1 (pond)	0.400	0.055	0.455	0.031
R1 (stream)	6.020	3.113	9.133	0.614
R3 (stream)	8.359	4.155	12.514	0.842
R4 (stream)	6.057	1.088	7.145	0.480

If the combined risk assessment presented by Applicant is not accepted, the further risk assessment should be considered on the MS level.

9.5.3 Overall conclusions

An overview of the mitigation measures needed for the active substance Aclonifen is provided in the table below.

FOCUS scenario	Aclonifen			
	1.5 L/ha	VFSMOD	2.0 L/ha	VFSMOD
D1 Ditch	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb	-	10m nsb + 75% drn OR 15m nsb + 50% drn	-
D1 Stream	5m nsb + 75% drn OR	-	5m nsb + 90% drn OR	-

	10m nsb + 50% drn OR 20m nsb		10m nsb + 50% drn	
D2 Ditch	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb	-	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn	-
D2 Stream	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb	-	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn	-
D3 Ditch	5m nsb + 75% drn OR 10m nsb + 50% drn OR 15m nsb	-	5m nsb + 75% drn OR 10m nsb + 50% drn OR 15m nsb + 50% drn	-
D4 Pond	None	-	None	-
D4 Stream	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb	-	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn	-
D5 Pond	None	-	None	-
D5 Stream	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb	-	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn	-
D6 Ditch	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb	-	5m nsb + 75% drn OR 10m nsb + 50% drn	-
R1 Pond	None	-	None	-
R1 Stream	20m vfs OR 10m vfs + 10m nsb + 50% drn (EPAT)	10m vfs + 10m nsb + 50% drn OR 10m vfs + 10m nsb (EPAT)	20m vfs OR 10m vfs + 75 drn (EPAT)	10m vfs + 10m nsb + 50% drn OR 10m vfs (EPAT)
R3 Stream	20m vfs OR 10m vfs + 50 drn (EPAT)	20m vfs	20m vfs + 50 drn OR 10m vfs + 75 drn (EPAT)	20m vfs + 50% drn OR 10m vfs (EPAT)
R4 Stream	20m vfs OR 10m vfs + 50 drn (EPAT)	10m vfs + 50% drn OR 10m vfs (EPAT)	10m vfs + 75 drn (EPAT)	10m vfs + 10m nsb + 50% drn OR 10m vfs (EPAT)

vfs: vegetated filter strip
nsb: no spray buffer

An overview of the mitigation measures needed for the active substance flufenacet is provided in the table below. The risk for the metabolites of flufenacet is acceptable at Step 1-2.

FOCUS scenario	**Flufenacet**	
	1.5 L/ha	**2.0 L/ha**

D1 Ditch	Unresolved	Unresolved
D1 Stream	None	Unresolved
D2 Ditch	Unresolved	Unresolved
D2 Stream	Unresolved	Unresolved
D3 Ditch	None	None
D4 Pond	None	None
D4 Stream	None	None
D5 Pond	None	None
D5 Stream	None	None
D6 Ditch	Unresolved	Unresolved
R1 Pond	None	None
R1 Stream	None	10m vfs
R3 Stream	10m vfs	10m vfs
R4 Stream	None	None

vfs: vegetated filter strip

The mitigation measures that are required based on the combined risk assessment are provided in the table below. FOCUS scenarios where the risk remains unresolved for one or both of the active substances have been excluded.

FOCUS scenario	**Combined toxicity (AGD Stepwise approach)**			
	1.5 L/ha	**1.5 L/ha (VFSMOD)**	**2.0 L/ha**	**2.0 L/ha (VFSMOD)**
D1 Ditch	Unresolved	Unresolved	Unresolved	-
D1 Stream	None	None	Unresolved	-
D2 Ditch	Unresolved	Unresolved	Unresolved	-
D2 Stream	Unresolved	Unresolved	Unresolved	-
D3 Ditch	90% drn OR 5m nsb + 50% drn OR 10m nsb	-	90% drn OR 5m nsb + 50% drn OR 10m nsb	-
D4 Pond	75% drn OR 5m nsb	-	75% drn OR 5m nsb	-
D4 Stream	90% drn OR 5m nsb + 50% drn OR 10m nsb	-	90% drn OR 5m nsb + 75% drn OR 10m nsb + 50% drn	-
D5 Pond	75% drn OR 5m nsb	-	75% drn OR 5m nsb	-
D5 Stream	90% drn OR 5m nsb + 50% drn OR 10m nsb	-	5m nsb + 75% drn OR 10m nsb + 50% drn	-
D6 Ditch	Unresolved	-	Unresolved	-
R1 Pond	75% drn OR 5m nsb	-	75% drn OR 5m nsb	-
R1 Stream	10m vfs	10m vfs	20m vfs	10m vfs + 50% drn
R3 Stream	20m vfs	10m vfs + 50% drn	20m vfs	20m vfs
R4 Stream	10m vfs	10m vfs	20m vfs	10m vfs

drn: drift reduction nozzles
vfs: vegetated filter strip
nsb: no spray buffer

Taking into account the information presented above for both active substance and the combined risk assessment done following step approach and using the AGD excel tool the following mitigation measures are required to protect aquatic organisms.

The risk assessment of flufenacet is unsolved in the D1, D2 and D6; however, none of these scenarios is relevant in the Central Zone. Therefore, no mitigation measures or restrictions related to these scenarios are necessary.

FOCUS scenario	**Conclusions**			
	1.5 L/ha	**1.5 L/ha (VFSMOD)**	**2.0 L/ha**	**2.0 L/ha (VFSMOD)**
D3 Ditch	5m nsb + 75% drn OR 10m nsb + 50% drn	-	5m nsb + 75% drn OR 10m nsb + 50% drn	-
D4 Pond	5m nsb	-	5m nsb	-
D4 Stream	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb	-	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn	-
D5 Pond	5m nsb	-	5m nsb	-
D5 Stream	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb	-	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn	-
R1 Pond	5m nsb	-	5m nsb	-
R1 Stream	10m vfs + 50% drn	10m vfs	20m vfs	10m vfs + 50% drn
R3 Stream	20m vfs	20m vfs	20m vfs + 50% drn	20m vfs + 50% drn
R4 Stream	10m vfs + 50% drn	10m vfs	20m vfs	10m vfs

Considering the relevant scenarios in each MS, conclusions per country are done too:

Country	**1.5L/ha**		**2.0L/ha**	
	Landscape Mitigation Report (Step4)	**VFSMOD**	**Landscape Mitigation Report (Step4)**	**VFSMOD**
Poland (D3, D4, R1)	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 10m vfs + 50% drn	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 10m vfs	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 20m vfs	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 10m vfs + 50% drn
Belgium (D3, D4, R1)	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 10m vfs + 50% drn	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 10m vfs	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 20m vfs	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 10m vfs + 50% drn
Czech Republic (D4, R1)	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn

	In run-off susceptible soils: 10m vfs + 50% drn	In run-off susceptible soils: 10m vfs	In run-off susceptible soils: 20m vfs	In run-off susceptible soils: 10m vfs + 50% drn
Hungary (D3, D5, R1, R3, R4)	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 20m vfs	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 20m vfs	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 20m vfs + 50% drn	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 20m vfs + 50% drn
Ireland (D4)	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb	-	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn	-
Romania (D4, D5, R1, R3, R4)	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 20m vfs	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 20m vfs	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 20m vfs + 50% drn	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 20m vfs + 50% drn
Slovakia (D4, D5, R1)	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 10m vfs + 50% drn	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 10m vfs	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 20m vfs	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 10m vfs + 50% drn
Slovenia (only drift, run-off is not relevant)	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb	-	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn	-

drn: drift reduction nozzles
vfs: vegetated filter strip
nsb: no spray buffer

The conclusions for Germany are presented in the German National addendum.

zRMS Comments:

Aclonifen

The first tier risk assessment for aclonifen was performed based on the EU agreed endpoints and FOCUS Step 1 to 4 PECsw values.

For the application rate of 1.5L formulation/ha (810 g aclonifen/ha), based on the PEC FOCUS Step 4 values the risk is accepted for all scenarios when the appropriate risk mitigation measures have been applied.

For the application rate of 2L formulation/ha (1080 g aclonifen/ha), based on the PEC FOCUS Step 4

values the risk is acceptable for scenarios D1 Ditch, D1 Stream, D2 Ditch, D2 Stream, D3 Ditch, D4 Pond, D4 Stream, D5 Pond, D5 Stream, D6 Ditch, R1 Pond, R1 Stream, R3 Stream when the appropriate risk mitigation measures have been applied. In the case of R4 Stream, the risk is unacceptable.

In addition, for the refinement the PEC/RAC calculations with VFSMod was presented. The use of VFSMod should be considered at the Member State level.
When this approach is accepted the risk is acceptable for:

- for the application rate of 1.5L formulation/ha (810 g aclonifen/ha)
- R1 pound with 10 m vegetative strip,
- R1 stream with 10 m vegetative strip and 50% drift reduction or 20 m vegetative strip,
- R3 stream with 20 m vegetative strip,
- R4 stream with 10 m vegetative strip and 50% drift reduction or 20 m vegetative strip.

- for the application rate of 2L formulation/ha (1080 g aclonifen/ha)
- R1 pound with 10 m vegetative strip,
- R1 stream with 10 m vegetative strip and 50% drift reduction or 20 m vegetative strip,
- R3 stream with 20 m vegetative strip and 50% drift reduction,
- R4 stream with 10 m vegetative strip and 50% drift reduction or 20 m vegetative strip.

In order to refine the chronic risk to aquatic organisms the Applicant conducted the an Exposure Pattern Analysis (EPAT). EPAT is an evaluation tool specifically designed for the analysis of TOXSWA output files from the surface water model FOCUS TOXSWA, what is currently not analysed further. The EPAT analysed how often a given concentration is reached or how long a concentration may be expected to remain above a given threshold. https://rifcon.de/wp-content/uploads/2018/08/rifcon_epat_2010.pdf

The Exposure Pattern Analysis has been assessed in Part B8 and can be considered as supporting information. This should be considered at the Member State level.

Flufenacet and metabolites
The first tier risk assessment for flufenacet was performed based on the EU agreed endpoints and FOCUS Step 1 to 4 PECsw values. Based on the PEC FOCUS Step 4 values for Flufenacet the risk is acceptable for scenarios D3 Ditch, D4 Pond, D4 Stream, D5 Pond, D5 Stream, , R1 Pond, R1 Stream, R3 Stream and R4 Stream when the appropriate risk mitigation measures have been applied. In the case of D1 Ditch, D1 Stream, D2 Ditch, D2 Stream and D6 Ditch the risk is unacceptable.
The first tier risk assessment for metabolites of flufenacet was performed based on the EU agreed endpoints and FOCUS Step 1 to 2 PECsw values For metabolites the risk is acceptable.

GLOB1310aH

For GLOB1310aH, tests on invertebrates *Daphnia magna*, on algae *Pseudokirchneriella subcapitata*, *Desmodesmus subspicatus*, and on aquatic plants *Lemna gibba* and *Myriophyllum spicatum* were provided by Applicant, but no tests are reported for fish.
According to the Commission Regulation (EU) No 284/2013, point 10.2.1 Acute toxicity:
" *Test shall be carried out on one species from each of the three/four groups of aquatic organisms, that is to say fish, aquatic invertebrates, algae…*"
and
"*Testing shall be performed where:*
(a) the acute toxicity of the plant protection product cannot be predicted on the basis of the data for the active substance;.."

Acute toxicity data for active substance and formulation

Species	**Aclonifen**	**Flufenacet**	**GLOB1310aH**
	Endpoint	**Endpoint**	**Endpoint**
Oncorhynchus mykiss	>0.67 mg a.s./L	2.13 mg a.s./L	-

Daphnia magna	1.2 mg a.s./L	30.9 mg a.s./L	6.12 mg product/L corresponding to 2.71 mg aclonifen./L and 0.307 mg flufenacet/L
Pseudokirchneriella subcapitata	-	0.0045 mg a.s./L	0.0542 mg product/L corresponding to 0.0237 mg aclonifen./L and 0.00292 mg flufenacet/L
Desmodesmus subspicatus	0.0069 mg a.s,/L	-	0.1038 mg product/L corresponding to 0.04482 mg aclonifen./L and 0.00628 mg flufenacet/L
Lemna gibba	0.012 mg a.s./L	0.00243 mg a.s./L	Frond - 0.0116 mg product/L corresponding to 0.00496 mg aclonifen./L and 0.00074 mg flufenacet/L Biomass - 0.00362 mg product/L corresponding to 0.00155 mg aclonifen./L and 0.00024 mg flufenacet/L
Myriophyllum spicatum	-	-	0.3019 mg product/L corresponding to 0.1325 mg aclonifen./L and 0.0162 mg flufenacet/L

Based on the aquatic acute data for active substances is not expected that the formulation to be more sensitive to fish than algae and macrophytes. Moreover from the combined risk assessment it is clear that the toxicity driver of the toxicity to fish is Aclonifen. It can be assumed that acute toxicity to fish for formulation can be predicted on the basis of the data for the active. Additionally, taking into consideration Article 62 of Regulation 1107/2009 and because of animal welfare regarding to studies on vertebrates, no acute fish study with the formulation is considered necessary.

For the combined risk assessment two approaches are conducted. The first is based on the comparison of the toxicity of the formulation and the PECdrift. The second is based on the stepwise approach according to the decision scheme (EFSA Journal 2013;11(7):3290) using the tool AGD_AquaMix_v1.15.
The risk is acceptable than the appropriate risk mitigation measures were applied.

Based on the risk assessment for both active substances and the combined risk assessment the risk mitigation measures are required. The risk to flufenacet is unresolved for D1, D2 and D6 scenarios but these scenarios are irrelevant in the Central Zone.

The appropriate risk mitigation measures should be considered at national level. If it is necessary Member states will need to further consider the risk to aquatic organisms based on national requirements.

In conclusion, according to the performed risk assessment there is no potential of risk for aquatic organisms resulting from acute and long-term exposure to active substances following use of **Glosset Ace (GLOB1310aH)** in compliance with proposed GAP when the risk mitigation measures were applied.

9.6 Effects on bees (KCP 10.3.1)

9.6.1 Toxicity data

Studies on the toxicity to bees have been carried out with Aclonifen. Full details of these studies are provided in the respective EU DAR and related documents as well as in 0 of this document (new studies).

Effects on bees of GLOB1310aH were not evaluated as part of the EU assessment of Aclonifen. New data submitted with this application are listed in Appendix 1 and summarised in 0.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

Table 0-1: Endpoints and effect values relevant for the risk assessment for bees

Species	Substance	Exposure System	Results	Reference
Apis mellifera	Aclonifen	Acute Oral, 48h	LD_{50} > 107 µg/bee	EFSA, 2008
		Acute Contact, 48h	LD_{50} > 100 µg/bee	EFSA, 2008
	Flufenacet	Acute Oral	LD_{50} > 170 µg/bee	Review report 2003
		Acute Contact	LD_{50} > 194 µg/bee	Review report 2003
	GLOB1310aH	Acute Oral	LD_{50} > 2000 µg/bee (>977 µg total a.s./bee)	Franke M., 2021 (21 48 BAA 0001)
		Acute Contact	LD_{50} > 2000 µg/bee (>977 µg total a.s./bee)	
	GLOB1310aH	Chronic adult, 10d	NOED = 43.7 µg product/bee/day (equivalent to 19.2 µg aclonifen/ bee/day, 2.13 µg flufenacet/ bee/day, and 21.33 µg total as/bee/day) 10d LDD_{50} > 193 µg product/bee/day (equivalent to > 84.9 µg aclonifen/ bee/day, >9.43 µg flufenacet/ bee/day, 94.33 µg total as/bee/day)	Dressler K., 2021 (21 48 BAC 0004)
	GLOB1310aH	Chronic, honeybee larvae, 22d	NOED = 29.6 µg product/larva (equivalent to 13.1 µg aclonifen/ larva, 1.48 µg flufenacet/ larva, and 14.58 µg total as/larva*)	Hänsel, M., 2021 (21 48 BKC 0003)
Bombus terrestris	GLOB1310aH	Acute Oral, 48h	LD_{50} > 716.9 µg/bee (>350.2 µg total a.s./bee)	Amsel, K., 2021 (21 48 BBA 0001)

Species	Substance	Exposure System	Results	Reference
		Acute Contact, 48h	LD_{50} > 818.8 µg/bumblebee (>400.0 µg total a.s./bee)	

9.6.1.1 Justification for new endpoints

As GLOB1310aH is not identical to the reference formulation used during the EU Review of Aclonifen and Flufenacet, toxicity to bees from the formulation was also tested and used in the risk assessment.

9.6.2 Risk assessment for honeybess

The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use 2L GLOB1310aH/ha also covers the risk for honey bees from all other intended uses.

Applications of pesticides can potentially result in exposure of honeybees either through direct overspray, or by contact with residues on plants whilst bees are foraging for food.

The risk assessment is carried out for the use in cereals as this is the only use proposed. The maximum application rate for GLOB1310aH is 2L/ha corresponding to 1080 g Aclonifen /ha and 120 g Flufenacet/ha.

9.6.2.1 Hazard quotients for bees – acute toxicity

The acute risk to honeybees from use of GLOB1310aH was assessed using the maximum single application rate and the LD_{50} values to calculate hazard quotients ***(*EPPO 2010*)*** as follows:

$$Hazard\ Quotient = \frac{Maximum\ application\ rate\ (g\ formulation/ha)}{Acute\ LD_{50}\ (\mu g\ formulation/bee)}$$

Hazard quotients were calculated for oral exposure (Q_{HO}) and contact exposure (Q_{HC}) to GLOB1310aH. A hazard quotient of less than 50 indicates a low risk to bees in the field.

Table 0-2: First-tier assessment of the risk for bees due to the use of GLOB1310aH in cereals

Intended use	Cereals		
Active substance	Aclonifen		
Application rate (g/ha)	1 x 1080 g/ha		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	> 107	1080	<10.09

Contact toxicity	> 100		<10.8
Active substance **Application rate (g/ha)**	Flufenacet 1 x 120 g/ha		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>170	120	0.71
Contact toxicity	>194		0.62
Product **Application rate (g/ha)**	GLOB1310aH 1 x 2456.4 g/ha(A)		
Test design	**LD_{50} (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q_{HO}, Q_{HC} criterion: $Q_H \leq 50$**
Oral toxicity	>2000	2456.4	<1.23
Contact toxicity	> 2000		<1.23

Q_{HO}, Q_{HC}: Hazard quotients for oral and contact exposure. Q_H values shown in bold breach the relevant trigger.
(A) Proposed use rate is 2 L formulation/ha, equivalent to 2.4564 kg based on a density of 1..2282 g/mL

All the hazard quotients are below 50, indicating that GLOB1310aH poses a low risk to bees. Therefore, a low acute risk to bees is expected from the application of GLOB1310aH.

9.6.2.2 Chronic risk assessment

Adult honeybee

The chronic risk to bees has been assessed following the **EPPO 2010 scheme**[2], as proposed in the list of guidance documents relevant to the implementation of Regulation 1107/2009, published in the official EU Journal 2013/C 95/01 and 95/02.

The EPPO 2010 scheme does not recommend a chronic assessment for adults for foliar spray applications. Moreover, the risk assessment scheme presented in EPPO 2010 is intended for seed treatment. A modified risk assessment of the EPPO 2010 (modified by ECPA 2017) is used here in order to assess the chronic risk to adult honeybees:

TER= NOEDD / Daily dose

Where daily dose is based on the worst case sugar need of 128 mg/bee/day (Rortais *et al*., 2005) of a bee feeding exclusively from nectar containing a more representative 30% sugar (EFSA, 2013) using the following equation:

Daily dose (µg a.s./bee/day) = A.R. x (0.128 g/(1000 x 0.3)) x RUD x 1000
A.R. = application rate in kg a.s./ha
RUD = residue per unit dose from EFSA (2013). Mean RUDnectar = 2.9 mg a.s./kg (foliar sprays)

The TER trigger is 1.

The risk assessment is conducted considering the highest intended application rate of GLOB1310aH, namely 2L/ha (1080 g Aclonifen/ha + 120 g Flufenacet/ha, total of 1200 g active substance/ha).

[2] EPPO/OEPP (2010). Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees (PP 3/10(3)). Bulletin OEPP/EPPO Bulletin 40: 323-331.

Daily Dose (µg a.s./bee/day)= 1.2 x (0.128 g/(1000 x 0.3)) x 2.9 x 1000= 0.534

NOED 21.33 µg total a.s./bee/day (according to study conducted with the formulation, Dressler K., 2021, 21 48 BAC 0004)

TER= 21.33 / 0.534 = 14.36

The EPPO 2010 scheme modified by ECPA 2017 proposes a trigger of 1 for assessment of the risk to honey bees. It is clear that with a TER value of 14.36, there is a wide safety margin, indicating that the proposed uses of GLOB1310aH pose an acceptable chronic risk to adult bees.

Larva - honeybee

The chronic risk to bees has been assessed following the EPPO 2010 scheme, as proposed in the list of guidance documents relevant to the implementation of Regulation 1107/2009, published in the official EU Journal 2013/C 95/01 and 95/02.

A chronic larval study is available and the potential acceptable risk can be further demonstrated by carrying out a worst-case risk assessment through the calculation of a TER value as set out in the EPPO 2010 scheme (point 5, on the scheme).

A worst-case of potential exposure via residues in pollen and nectar can be estimated based on the default worst-case residue of 1 mg a.s./kg proposed in the EPPO 2010 scheme (see Note 6), based on a database of measured values from aerial plant parts, as a surrogate for nectar and pollen.

The default residues can then be combined with a measure of consumption in order to estimate the exposure. Worst case data from ***Rortais et al., 2005***[3], as proposed in the EPPO scheme, have been used to estimate the consumption by bee larvae:

Worst case: drone larvae consuming 98.2 mg sugar in 6.5 days (= 15.1 mg sugar /day).

Thus, considering residues of 1 mg a.s./kg sugar x consumption of 15.1 mg sugar/bee/day

Total exposure ETE = 0.0151 µg a.s./bee/day

This can be compared to the GLOB1310aH larval NOED of 14.58 µg a.s./larva, which is 0.66 µg a.s./larva/day (based on 22 day study duration).

- TER = NOED (µg a.s./larva/day)/ ETE (µg a.s./bee/day)

  = 0.66/0.0151 = 43.7

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to honey bees. It is clear that with a TER value of 43.7 there is a wide safety margin, indicating that the proposed uses of GLOB1310aH poses an acceptable risk to bee larval development

Risk assessment following EFSA Guidance

Screening assessment

[3] Agnès RORTAIS, Gérard ARNOLD, Marie-Pierre HALM, Frédérique TOUFFET-BRIENS (2005). Modes of honeybees exposure to systemic insecticides: estimated amounts of contaminated pollen and nectar consumed by different categories of bees. Apidologie 36 (2005) 71–83

Table 9.6-3: Screening assessment - acute risk to adult honeybees from contact exposure

Crop	Single application rate (g a.s./ha)	Species (life stage)	LD_{50} (µg a.s./bee)	$HQ_{contact}$	Trigger value
Cereals	2456.4	Honeybee (adult)	> 2000	< 1.2	42
		Bumble bee	> 818.8	< 3.0	7

HQ: Hazard quotient. HQ values shown in **bold** breach the relevant trigger, indicating potential concern. SUW: sideward/upward spray; DW: downward spray.

The above screening risk assessment demonstrates an acceptable risk to honeybees from acute contact exposure from all proposed uses of GLOB1310aH.

Table 9.6-4: Screening assessment - acute risk to adult honeybees from oral exposure

Crop	Single application rate (kg a.s./ha)	Species (life stage)	LD_{50} (µg a.s./bee)	Ef x SV (downward)	ETR_{oral}	Trigger value
Cereals	2.4564	Honeybee (adult)	> 2000	7.6	< 0.01	> 0.2

SV: Shortcut value; ETR: Exposure toxicity ratio. ETR values shown in **bold** are greater than the relevant trigger, indicating potential concern. SUW: sideward/upward spray; DW: downward spray.

The above screening risk assessment demonstrates an acceptable risk to honeybees from acute oral exposure from all proposed uses of GLOB1310aH.

Table 9.6-5: Screening assessment - chronic risk to adult honeybees and honeybee larvae from oral exposure

Crop	Single application rate (kg a.s./ha)	Species (life stage)	Endpoint	Ef x SV (downward)	ETR_{oral}	Trigger value
Cereals	2.4564	Honeybee (adult)	LDD_{50} > 193 µg prod/bee	7.6	**< 0.097**	> 0.03
		Honeybee (larvae)	NOED = 29.6 µg product/larva	4.4	**0.37**	> 0.2

SV: Shortcut value; ETR: Exposure toxicity ratio. ETR values shown in **bold** are greater than the relevant trigger, indicating potential concern.

In the above screening risk assessment, an acceptable risk could not be demonstrated for adult honeybees or honeybee larvae, for any of the proposed uses of GLOB1310aH. Therefore, a first-tier risk assessment is provided.
Hence, a Tier 1 risk assessment for the scenarios bare soil, cereals BBCH <10 is presented below.

Table 0-6: Tier-I assessment of the chronic oral risk for adult bees due to the use of GLOB1310aH in winter cereals according to the new bee guidance EFSA Journal 2013; 11(7):3295 – Bare soil and Cereals BBCH <10 scenarios

Product	GLOB1310aH

Application rate (g/ha)	2456.4					
LD_{50}-10 d	193 µg prod/bee					
Intended use	**Bare soil**					
Test design	**Single application rate (kg prod/ha)**	**Ef**	**SV Downward**	**twa**	**ETR**	**Trigger value**
Risk from foraging on the treated crop						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	1	0.012[1)]	0.72	0.000	< 0.03
Risk from foraging on an adjacent crop						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	0.0033	5.8[1)]	0.72	< 0.000	< 0.03
Risk from foraging on weeds in the treated field						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	1	0.27	0.72	0.002	< 0.03
Risk from foraging in the field margin						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	0.0092	2.9	0.72	0.000	< 0.03
Risk from foraging the following year on a permanent crop or on a succeeding crop for annual crops						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	1	0.54[1)]	0.72	0.005	< 0.03
Intended use	**Cereals BBCH <10**					
Risk from foraging on the treated crop						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	1	0.012[1)]	0.72	< 0.000	< 0.03
Risk from foraging on an adjacent crop						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	0.0033	5.8[1)]	0.72	< 0.000	< 0.03
Risk from foraging on weeds in the treated field						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	1	2.9	0.72	0.027	< 0.03
Risk from foraging in the field margin						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	0.0092	2.9	0.72	0.000	< 0.03
Risk from foraging the following year on a permanent crop or on a succeeding crop for annual crops						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	1	0.54[1)]	0.72	0.005	< 0.03

ETR: Exposure toxicity ratio.

1) Treated crop – application before emergence (downward spraying) on crop attractive for pollen, only (cereals flower does not produce nectar).

Table 0-7: Tier-I assessment of the chronic oral risk for honeybee (larvae) due to the use of GLOB1310aH in winter cereals according to the new bee guidance EFSA Journal 2013; 11(7):3295 – Bare soil scenario

Product	GLOB1310aH					
Application rate (g/ha)	2456.4					
NOED	29.6 µg product/larva					
Intended use	**Bare soil**					
Test design	**Single application rate (kg prod/ha)**	**Ef**	**SV Downward**	**twa**	**ETR**	**Trigger value**
Risk from foraging on the treated crop						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	1	0.002[1)]	0.85	0.00	0.2
Risk from foraging on an adjacent crop						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	0.0033	4.4[1)]	0.85	0.00	0.2
Risk from foraging on weeds in the treated field						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	1[2)]	0.2	0.85	0.01	0.2
Risk from foraging in the field margin						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	0.0092	2.2	0.85	0.00	0.2
Risk from foraging the following year on a permanent crop or on a succeeding crop for annual crops						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	1	0.4[1)]	0.85	0.03	0.2
Intended use	**Cereals BBCH <10**					
Risk from foraging on the treated crop						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	1	0.002[1)]	0.85	0.00	0.2
Risk from foraging on an adjacent crop						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	0.0033	4.4[1)]	0.85	0.00	0.2
Risk from foraging on weeds in the treated field						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	1[2)]	2.2	0.85	0.16	0.2
Risk from foraging in the field margin						

Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	0.0092	2.2	0.85	0.00	0.2
Risk from foraging the following year on a permanent crop or on a succeeding crop for annual crops						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	1	0.4[1)]	0.85	0.03	0.2

ETR: Exposure toxicity ratio. ETR values shown in bold breach the relevant trigger.
1) Treated crop – application before emergence (downward spraying) on crop attractive for pollen, only (cereals flower does not produce nectar).

In the above Tier-1 risk assessment, an acceptable risk could be demonstrated for adult honeybees or honeybee larvae, for any of the proposed uses of GLOB1310aH.

9.6.3 Higher tier risk assessment for bees (tunnel test, field studies)

Not relevant

9.6.4 Effects on bumble bees

The maximum application rate for GLOB1310aH is 2L/ha corresponding to 1080 g Aclonifen /ha and 120 g Flufenacet/ha (Total of 1,200 g a.s./ha)

The evaluation of the risk for bumblebees was performed based on the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).

Table 0-8: First-tier assessment of the risk for bumble bees due to the use of GLOB1310aH

Product	GLOB1310aH		
Application rate (g/ha)	1 x 2456.4 g/ha[(A)]		
Test design	**LD$_{50}$ (lab.) (µg/bee)**	**Single application rate (g/ha)**	**Q$_{HO}$, Q$_{HC}$ criterion: Q$_H$ ≤ 50**
Oral toxicity	716.9	2456.4	3.42
Contact toxicity	818.8		<3.0

Q$_{HO}$, Q$_{HC}$: Hazard quotients for oral and contact exposure. Q$_H$ values shown in bold breach the relevant trigger.
(A) Proposed use rate is 2 L formulation/ha, equivalent to 2.4564 kg based on a density of 1..2282 g/mL

All the hazard quotients are below 50, indicating that GLOB1310aH poses a low risk to bumble bees. Therefore, a low acute risk to bees is expected from the application of GLOB1310aH.

Risk assessment following EFSA Guidance

Screening assessment

Table 9.6-9: Screening assessment - acute risk to bumble bee from contact exposure

Crop	**Single application rate**	**Species (life stage)**	**LD$_{50}$ (µg a.s./bee)**	**HQ$_{contact}$**	**Trigger value**

	(g a.s./ha)				
Cereals	2456.4	Bumble bee	> 818.8	< 3.0	7

HQ: Hazard quotient. HQ values shown in **bold** breach the relevant trigger, indicating potential concern. SUW: sideward/upward spray; DW: downward spray.

The above screening risk assessment demonstrates an acceptable risk to honeybees from acute contact exposure from all proposed uses of GLOB1310aH.

Table 9.6-10: Screening assessment - acute risk to adult bumble bee from oral exposure

Crop	Single application rate (g a.s./ha)	Species (life stage)	LD_{50} (µg a.s./bee)	Ef x SV (downward)	ETR_{oral}	Trigger value
Cereals	2456.4	Bumble bee	> 716.9	11.2	**< 0.04**	> 0.036

SV: Shortcut value; ETR: Exposure toxicity ratio. ETR values shown in **bold** are greater than the relevant trigger, indicating potential concern. SUW: sideward/upward spray; DW: downward spray.

The above screening risk assessment do not demonstrates an acceptable risk to bumble bee from acute oral exposure from all proposed uses of GLOB1310aH.
Hence, a Tier 1 risk assessment for the scenarios bare soil, cereals BBCH <10 is presented below.

Table 0-11: Tier-I assessment of the acute oral risk for bumble bee due to the use of GLOB1310aH in winter cereals according to the new bee guidance EFSA Journal 2013; 11(7):3295 – Bare soil and Cereals BBCH <10 scenarios

Product	GLOB1310aH					
Application rate (g/ha)	2456.4					
LD_{50}-10 d	716.9µg prod/bee					
Intended use	**Bare soil**					
Test design	**Single application rate (kg prod/ha)**	**Ef**	**SV Downward**	**twa**	**ETR**	**Trigger value**
Risk from foraging on the treated crop						
Tier 1 ETR=AR*Ef*SV*twa/10dLD_{50}	2.4564	1	0.03[1)]	1	0.00	0.2
Risk from foraging on an adjacent crop						
Tier 1 ETR=AR*Ef*SV*twa/10dLD_{50}	2.4564	0.0033	11.2[1)]	1	0.00	0.2
Risk from foraging on weeds in the treated field						
Tier 1 ETR=AR*Ef*SV*twa/10dLD_{50}	2.4564	1	0.46	1	0.00	0.2
Risk from foraging in the field margin						
Tier 1 ETR=AR*Ef*SV*twa/10dLD_{50}	2.4564	0.0092	6.5	1	0.00	0.2

Risk from foraging the following year on a permanent crop or on a succeeding crop for annual crops						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	1	0.9[1)]	1	0.00	0.2
Intended use	**Cereals BBCH <10**					
Risk from foraging on the treated crop						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	1	0.03[1)]	1	0.00	0.2
Risk from foraging on an adjacent crop						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	0.0033	11.2[1)]	1	0.00	0.2
Risk from foraging on weeds in the treated field						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	1	6.5	1	0.00	0.2
Risk from foraging in the field margin						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	0.0092	6.5	1	0.00	0.2
Risk from foraging the following year on a permanent crop or on a succeeding crop for annual crops						
Tier 1 ETR=AR*Ef*SV*twa/10dLD$_{50}$	2.4564	1	0.9[1)]	1	0.00	0.2

ETR: Exposure toxicity ratio.
1) Treated crop – application before emergence (downward spraying) on crop attractive for pollen, only (cereals flower does not produce nectar).

In the above Tier-1 risk assessment, an acceptable risk could be demonstrated for bumble bee, for any of the proposed uses of GLOB1310aH.

9.6.5 Effects on solitary bees

Not required

9.6.6 Overall conclusions

The acute and long term risk to honey bees (adult and larvae) and to bumble bees is acceptable when following the proposed GAP of GLOB130aH.

Review comments:

The evaluation of the risk for bees was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002).

The required study on oral and contact toxicity of the formulated product GLOB1310aH to honeybees was conducted and considered to be valid.

The endpoints as proposed by the notifier are considered acceptable and are used in the risk assessment. All hazard quotients for acute oral and acute contact exposure were below 50, the Commission Regulation (EU) No. 546/2011 criterion, indicating low risk to honey bees.

The specific requirements of the Regulation (EU) 546/2011 regarding effects on bee brood development and possible chronic effects on adults were included by the Applicant.

The EPPO 2010 scheme proposes a trigger of 1 for assessment of the risk to honey bees. All TER values for chronic risk assessment for adult bees and bee larvae were above a trigger of 1, indicating that the proposed uses of GLOB1310aH poses an acceptable chronic risk to adult bees.

Moreover, the evaluator provided the risk assessment according to the new bee guidance "EFSA Guidance Document on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp.) and solitary bees", EFSA Journal 2013; 11(7):3295. For chronic oral exposure of adult bees, an unacceptable risk was indicated in the screening step however in the Tier 1 assessment, no unacceptable risk was indicated.

The acute and long term risk to honey bees (adult and larvae) and to bumble bees is acceptable when following the proposed GAP of GLOB130aH.

9.7 Effects on arthropods other than bees (KCP 10.3.2)

9.7.1 Toxicity data

Effects on non-target arthropods of GLOB1310aH were not evaluated as part of the EU assessment of aclonifen and flufenacet. New data submitted with this application are listed in 0 and summarised in 0.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

Table 0-1: Endpoints and effect values relevant for the risk assessment for non-target arthropods

Species	Substance	Exposure System	Results	Reference
Typhlodromus pyri	GLOB1310aH	Extended Laboratory test Bean laves (2D)	LR_{50} > 500 mL/ha ER_{50} > 500 mL/ha	Röhling U, 2021 (21 48 NTE 0008)
Aphidius rhopalosiphi	GLOB1310aH	Extended Laboratory test Potted barley plants (3D)	LR_{50} > 1500 mL/ha ER_{50} > 1500 mL/ha	Röhling U, 2021 (21 48 NAE 0007)
Poecilus cupreus	GLOB1310aH	Extended laboratory test	LR_{50} > 2000 mL/ha ER_{50} > 2000 mL/ha	Röhling U, 2021 (21 48 NLE 0001)

Species	Substance	Exposure System	Results	Reference
		Sandy soil (2D)		
Aleochara bilineata	GLOB1310aH	Extended laboratory test Sandy soil (2D)	ER_{50} > 2000 mL/ha	Röhling U, 2021 (21 48 NKE 0001)
Aphidius rhopalosiphi	GLOB1310aH	Aged-residue test Potted leave plants (3D)	Mortality (corrected): <50% at 0 DAT at 2L/ha <50% at 7 DAT at 2L/ha Reproduction (corrected): <50% at 0 DAT at 2L/ha <50% at 7 DAT at 2L/ha	Röhling U, 2021 (21 48 NAR 0001)
Typhlodromus pyri	GLOB1310aH	Aged-residue test Detached bean leaves (2D)	Mortality (corrected): <50% at 7 DAT at 2L/ha <50% at 14 DAT at 2L/ha Reproduction (corrected): <50% at 7 DAT at 2L/ha <50% at 14 DAT at 2L/ha	Röhling U, 2021 (21 48 NAR 0001)

9.7.1.1 Justification for new endpoints

As the formulation GLOB1310aH is not the same formulation as the one of the EU Review of aclonifen and flufenacet, toxicity to non-target arthropods from GLOB1310aH was tested and used in the risk assessment.

Tier 1 was not performed, because based on the knowledge the applicant has on Aclonifen, extended laboratory test would be needed for *Typhlodromus pyri* and *Aphidius rophalosipi*. Assuming then a failure of the risk assessment for the two mentioned species for both in-field and off-field, following recommendation of ESCORT I2, extended laboratory tests (higher tier) were performed for the species at risk, plus 2 additional species (i.e. here *Poecilus cupreus* and *Aleochara bilineata*).

As a risk a-was detected (in-field) during the extended laboratory test, an aged residue test was done on this species to show the potential of recolonisation.

9.7.2 Risk assessment

The evaluation of the risk for non-target arthropods was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev.2 (final), October 17, 2002), and in consideration of the recommendations of the guidance document ESCORT 2.

9.7.2.1 Risk assessment for in-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use of 2L GLOB1310aH/ha, the highest intended application rate, also covers the risk for non-target arthropods from all other intended uses.

Table 0-2: First- and higher-tier assessment of the in-field risk for non-target arthropods due to the use of GLOB1310aH in winter cereals

Intended use	Winter cereals
Active substance/product	GLOB1310aH
Application rate	1 x 2 L/ha
MAF	1

Test species Higher-tier (extended lab)	**Rate with ≤ 50 % effect* (L/ha)**	**$PER_{in-field}$ (L/ha)**	**$PER_{in-field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	>0.5	2	**No**
Aphidius rhopalosiphi	>1.5	2	**No**
Poecilus cupreus	>2	2	Yes
Aleochara bilineata	>2	2	Yes
Test species Higher-tier (aged residues)	**Rate with ≤ 50 % effect (L/ha) at 7 DAT**	**$PER_{in-field}$ (g/ha)**	**$PER_{in-field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	Mortality and reproduction >2 L at 7 DALT	2	Yes
Aphidius rhopalosiphi	Mortality and reproduction >2 L at 7 DALT	2	Yes

MAF: Multiple application factor; PER: Predicted environmental rate; HQ: Hazard quotient; DALT: Days after last treatment.
Criteria values shown in bold breach the relevant trigger.

The in-field HQ value for *Typhlodromus pyri* and *Aphidius ropalosiphi* are above the ESCORT 2 trigger value of 1 for Tier II (higher tier tests), indicating a potential risk. However, aged residue testing showed that after 7 days, no effects > 50 % were observed at a dose of 2 L/ha (ER50 >2L/ha), demonstrating the potential for in-field recovery of *Typhlodromus pyri* and *Aphidius ropalosiphi* in seven days. According to ESCORT2, a potential of recolonisation in-field has to be demonstrated within one year.

Tier II tests were conducted on the soil-dwelling species *Aleochara bilineata* and *Poecilus cupreus*, in which no effects on parasitism rate of *Aleochara bilineata* were observed, no effects on survival of *Poecilus cupreus*, and no sublethal effects for both species, indicating that the in-field risk for these two soil-dwelling species is acceptable.

It is concluded that the in-field risk of the proposed use of GLOB1310aH to non-target arthropods is acceptable following application according to the proposed use patterns.

9.7.2.2 Risk assessment for off-field exposure

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use 2L GLOB1310aH/ha, the highest intended application rate, also covers the risk for non-target arthro-

pods from all other intended uses.

Table 0-3: First- and higher-tier assessment of the off-field risk for non-target arthropods due to the use of GLOB1310aH in winter cereals (2L GLOB1310aH/ha)

Intended use	Winter cereals
Active substance/product	GLOB1310aH
Application rate (L/ha)	1 × 2L/ha
MAF	1
vdf	~~10~~ 5 (2D) / 1 (3D)

Test species Tier I	**LR_{50} (lab.) (gL/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (gL/ha)**	**CF**	**$HQ_{off\text{-}field}$ criterion: HQ ≤ 2**
Typhlodromus pyri	>0.5	2.77%	~~0.001108~~ 0.00554 0.277	5	~~0.00221~~ 0.0554
Aphidius rhopalosiphi	>1.5				~~0.00073~~ 0.185
Test species Higher-tier	**Rate with ≤ 50 % effect* (L/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (L/ha) 0.001108**	**CF**	**corr. $PER_{off\text{-}field}$ below rate with ≤ 50 % effect?**
Typhlodromus pyri	>0.5	2.77%	~~0.001108~~ 0.00554	5	yes
Aphidius rhopalosiphi	>1.5	2.77%	~~0.001108~~ ~~0.00554~~ 0.277	5	yes

MAF: Multiple application factor; vdf: Vegetation distribution factor; (corr.) PER: (corrected) Predicted environmental rate; CF: Correction factor; HQ: Hazard quotient. Criteria values shown in bold breach the relevant trigger.

* If an LR_{50} or ER_{50} from a relevant extended laboratory test is available, it should be considered in place of the rate with ≤ 50 % effect.

9.7.2.3 Additional higher-tier risk assessment

Not relevant.

9.7.2.4 Risk mitigation measures

No risk mitigation needed.

9.7.3 Overall conclusions

The in- and off-field risks for the non-target arthropods *Aphidius rhopalosiphi*, *Typhlodromus pyri*, *Aleochara bilineata* and *Poecilus cupreus* are acceptable for the intended use in winter cereals as recommended in the GAP of GLOB1310aH.

Review comments:

The endpoints as proposed by the notifier are considered acceptable and are used in the risk assessment.

Risk for other arthropods species was assessed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev. 2 (final), October 17, 2002) and ESCORT 2.
The risk assessment indicated that no unacceptable adverse effects on non-target arthropods are to be expected for the in- or off-field habitats following the use of the product according to the proposed use pattern.

9.8 Effects on non-target soil meso- and macrofauna (KCP 10.4)

9.8.1 Toxicity data

Studies on the toxicity to earthworms and other non-target soil organisms (meso- and macrofauna) have been carried out with aclonifen and flufenacet and its relevant metabolites. Full details of these studies are provided in the respective EU DAR and related.

Effects on earthworms and other non-target soil organisms (meso- and macrofauna) of GLOB1310aH were not evaluated as part of the EU assessment of aclonifen and flufenacet. New data submitted with this application are listed in 0 and summarised in 0.

Table 0-1: Endpoints and effect values relevant for the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna)

Species	Substance	Exposure System	Results	Reference
Earthworm acute				
Eisenia fetida	Aclonifen	Mixed into substrate 14 d, acute 10 % peat content	LC_{50} = 300 mg/kg dw $LC_{50,corr}$ = 150 mg a.s./kg dw*	EFSA, 2008
Eisenia fetida	Flufenacet	14 d, acute	LC_{50} = 219 mg met/kg dw	Review Report, 2003
Eisenia fetida	FOE-sulfonic acid	14 d, acute	LC_{50} >1000 mg met/kg dw	Review Report, 2003
Eisenia fetida	FOE-oxalate	14 d, acute	LC_{50} >1000 mg met/kg dw	Review Report, 2003
Earthworm chronic				
Eisenia fetida	Aclonifen (tested as BANDUR SC 600 g/L)	28 d, chronic	Reproduction: NOEC = 45 mg a.s./kg dw soil*	EFSA, 2008
Eisenia fetida	Flufenacet	56 d, chronic (artificial subsrate)	NOEC = 4 mg a.s./kg dw soil	Review Report, 2003
Eisenia fetida	GLOB1310aH	56d 10 % peat content	Mortality: NOEC = 204.1 mg a.s./kg dw soil NOECcorr = 102.05* mg a.s./kg dw soil	Friedrich S, 2021 (21 48 TEC 0003)

Species	Substance	Exposure System	Results	Reference
			Reproduction: NOECrepro = 63 mg a.s./kg dw soil NOECcorr = 31.5* mg a.s./kg dw soil	
Collembola				
Folsomia candida	GLOB1310aH	28 d, chronic 10 % peat content	Mortality: NOEC = 265.8 mg a.s./kg dw soil NOECcorr = 132.9* mg a.s./kg dw soil Reproduction: NOECrepro = 265.8 mg a.s./kg dw soil NOECcorr = 132.9* mg a.s./kg dw soil	Friedrich S, 2021 (21 48 TCC 0002)
Hypoaspis				
Hypoaspis aculeifer	GLOB1310aH	14 d, chronic 10 % peat content	Mortality: NOEC > 1020.4 mg a.s./kg dw soil NOECcorr = 510.2* mg a.s./kg dw soil Reproduction: NOECrepro = 566.9 mg a.s./kg dw soil NOECcorr = 283.45* mg a.s./kg dw soil	Schulz L, 2021 (21 48 THC 0001)

* Corrected value derived by dividing the endpoint by a factor of 2 in accordance with the EPPO earthworm scheme 2002.
** This study has been conducted according to the old guideline of Bakker et al. (2002) on a natural soil (therefore, no endpoint correction is necessary)

9.8.1.1 Justification for new endpoints

In the EU review of Aclonifen and Flufenacet respectively, there is no endpoint for *Folsomia candida* and *Hypoaspis aculeifer*. Therefore, the risk assessment is conducted with the endpoint from the formulation GLOB1310aH.

As GLOB1310aH is not identical to the reference formulation used during the EU Review of Aclonifen and Flufenacet, toxicity to soil macro-organisms from the formulation was also tested and used in the risk assessment. New data submitted with this application (on earthworm, *Folsomia* and *Hypoaspis*) are listed in 0 and summarised in 0.

9.8.2 Risk assessment

The evaluation of the risk for earthworms and other non-target soil organisms (meso- and macrofauna) was performed in accordance with the recommendations of the “Guidance Document on Terrestrial Ecotoxicology”, as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

9.8.2.1 First-tier risk assessment

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use 2L GLOB1310aH/ha, the highest intended application rate, also covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all other intended uses of GLOB1310aH.

Table 0-2: First-tier assessment of the acute and chronic risk for earthworms and other non-target soil organisms (meso- and macrofauna) due to the use of GLOB1310aH in winter cereals

Intended use	Winter cereals, pre-emergence 2L/ha		
Acute effects on earthworms			
Product/active substance	**LC_{50} (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_a (criterion TER ≥ 10)**
Aclonifen	150	1.575	95.2
Flufenacet	219 109.5*	0.160	1368 684.4
FOE-Sulfonic acid (M2)	1000	0.0524	19084
FOE-Oxalate (M1)	1000	0.0155	64516
Chronic effects on earthworms			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
Aclonifen (tested item BANDUR SC 600 g/L)	45*	1.575	28.6
Flufenacet	4 2*	0.160	25 12.5
GLOB1310aH	31.5	3.2752	9.6
Chronic effects on *Folsomia candida*			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
GLOB1310aH	132.9	3.2752	40.6
Chronic effects on *Hypoaspis aculeifer*			
Product/active substance	**NOEC (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 5)**
GLOB13101aH	283.45	3.2752	86.5

TER values shown in bold fall below the relevant trigger.
*endpoint has been corrected due to log Pow > 2.0

RMS comments:

Acute effect on earthworms is no longer required according to Regulation (EC) 1107/2009 but for completeness the acute endpoint for Flufenacet at 219 mg/kg dw has been corrected due to log Pow> 2.0 (see Table 9.8 2).

Exposure:
PECsoil values were calculated considering GAP of Glosset Ace. The highest predicted environmental

concentrations (PECsoil) of the active substances and formulation were taken into account for the risk assessment.

Toxicity data:
For the risk assessment the EU agreed endpoints were used for aclonifen (EFSA, 2008) and flufenaced (Review Report, 2003). For formulation the results of studies were used.

Earthworms
Aclonifen – according to the EFSA Scientific Report (2008) 149, the chronic risk was based on the result of study with BANDUR SC 600 g/L (reproduction NOECcorr at 45 mg as/kg dw soil). Therefore the TER calculation with this endpoint was performed by RMS above. The long-term TER value for the aclonifen is above the trigger value of 5.

Flufenacet – for the assessment of chronic effect on earthworm the EU agreed endpoint NOEC = 4 mg a.s./L was used. The log Pow for flufenacet is above 2, therefore this value should be dividing by a factor of 2. The corrected TER calculation was carried out (see Table 9.8 2). The long-term TER value for the flufenacet is above the trigger value of 5.

It should be noted that the renewal of flufenacet is ongoing.

GLOB1310aH/Glosset Ace – for the assessment of long-term risk to earthworms the corrected NOEC reproduction value from study with the formulation was used.
The long-term TER value for the formulation is above the trigger value of 5.

Other soil macro-organisms
The risk assessment was performed based on the endpoint from the formulation studies. But in EFSA Scientific Report (2008) for the chronic-risk for aclonifen the result of study with formulation BAN-DUR SC 600 g/L, expressed as mg a.s./kg dw soil, was used. Therefore the TER_{LT} calculation was provided below.

Intended use	Winter cereals, pre-emergence 2L/ha		
Chronic effects on Hypoaspis aculeifer EFSA (2008)			
active substance	**NOECmorality (mg aclonifen/kg dw soil)**	**PEC_{soil} (mg/kg dw)**	**TER_{lt} (criterion TER ≥ 1)**
Aclonifen (tested item BANDUR SC 600 g/L)	13.3	1.575	8.4

The long-term TER value for the aclonifen is above the trigger value of 5. Additionally, according to EFSA Scientific Report (2008) for aclonifen: "…the long-term risk to soil dwelling mites was considered to be low since reproduction was not affected and the morality was only 13% at the higher tested concen-tration of 42.1 mg a.s./kg soil."

All TER_{LT} values for formulation are above trigger value of 5.

Conclusion:
According to the performed risk assessment there is low chronic risk to earthworms and other non-target organisms resulting from long-term exposure to active substances following use of Glosset Ace (GLOB1310aH) in compliance with proposed GAP.

9.8.2.2 Higher-tier risk assessment

Not relevant.

9.8.3 Overall conclusions

All TER values exceed their respective triggers, indicating that GLOB1310aH poses a low acute and chronic risk to earthworms and low long-term risks to other soil macro- and mesofauna when applied according to the proposed uses.

9.9 Effects on soil microbial activity (KCP 10.5)

9.9.1 Toxicity data

Studies on effects soil microorganisms have been carried out with aclonifen, flufenacet and their relevant metabolites. Full details of these studies are provided in the respective EU DAR and related documents.

Effects on soil microorganisms of GLOB1310aH were not evaluated as part of the EU assessment of aclonifen and flufenacet. New data submitted with this application are listed in 0 and summarised in 0.

The selection of studies and endpoints for the risk assessment is in line with the results of the EU review process.

Table 0-1: Endpoints and effect values relevant for the risk assessment for soil microorganisms

Endpoint	Substance	Exposure System	Results	Reference
N-mineralisation	Aclonifen	56 d, sand and loam soil	22 % effect at day 28 at 20 mg a.s./kg dry soil	EFSA, 2008
	Flufenacet	28 d, aerobic Sandy loam soil	No effects >25% at 0.62 and 3.1 kg/ha (=0.8 and 4 mg a.s./kg dw soil)	Review Report, 2003
	FOE-sulfonic acid	28 d, aerobic Sandy loam soil	0.52 mg/kg soil and 5.2 mg/kg soil: no significant effect	Servajean 2014
	FOE-oxalate	28 d, aerobic Sandy loam soil	0.16 mg/kg soil and 1.60 mg/kg soil: no significant effects	Servajean, 2014
	GLOB1310aH	28 d, loamy sand	<25% effect at day 28 at 3.8 mg formulation per kg dry soil <25% effect at day 28 at 19 mg formulation per kg dry soil	Schulz L, 2021 (21 48 SMN 0001)
C-mineralisation	Aclonifen	28 d, sandy soil	8 % effect at day 28 at 4 mg a.s./kg dry soil	EFSA, 2008

Endpoint	Substance	Exposure System	Results	Reference
		28 d, loamy soil	25 % effect at day 28 at 20 mg a.s./kg dry soil	
	Flufenacet	28 d, aerobic Sandy loam soil	No effects >25% at 0.62 and 3.1 kg/ha (=0.8 and 4 mg a.s./kg dw soil)	Review Report, 2003
	FOE-sulfonic acid	28 d, aerobic Sandy loam soil	0.52 mg/kg soil and 5.2 mg/kg soil: no significant effect	Servajean 2014
	FOE-oxalate	28 d, aerobic Sandy loam soil	0.16 mg/kg soil and 1.60 mg/kg soil: no significant effects	Servajean 2014

9.9.1.1 Justification for new endpoints

As GLOB1310aH is a different formulation than the representative one used during the EU Review of Aclonifen and Flufenacet, toxicity to soil microorganisms from the formulation was also tested and used in the risk assessment.

9.9.2 Risk assessment

The evaluation of the risk for soil microorganisms was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

The relevant PEC_{soil} for risk assessments covering the proposed use pattern are taken from Section 8 (Environmental Fate), Chapter 8.7.2, and were already used in the risk assessment for earthworms and other non-target soil organisms (meso- and macrofauna) (see 0).

To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use 2L GLOB1310aH/ha, the highest intended application rate, also covers the risk for earthworms and other non-target soil organisms (meso- and macrofauna) from all other intended uses of GLOB1310aH.

Table 0-2: Assessment of the risk for effects on soil micro-organisms due to the use of GLOB1310aH in winter cereals (2L/ha)

Intended use	Winter cereals (2L/ha, pre-emergence)		
N-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Aclonifen	20 (at 28 d)	~~1.981~~ 1.575	Yes
Flufenacet	4 (at 28 d)	0.160	Yes
FOE-sulfonic acid	5.2 (at 28 d)	0.0524	Yes
FOE-oxalate	1.60 (at 28 d)	0.0155	Yes
GLOB1310aH	19.0 (at 28 d)	3.2752	Yes

C-mineralisation			
Product/active substance	**Max. conc. with effects ≤ 25 % (mg/kg dw)**	**PEC_{soil} (mg/kg dw)**	**Risk acceptable?**
Aclonifen	20 (at 28 d)	1.575	Yes
Flufenacet	4 (at 28 d)	0.160	Yes
FOE-sulfonic acid	5.2 (at 28 d)	0.0524	Yes
FOE-oxalate	1.60 (at 28 d)	0.0155	Yes

9.9.3 Overall conclusions

As the PEC_{soil} of Aclonifen, Flufenacet, its metabolites and the formulation are all lower than the concentration at which no significant effects are detected, it can be concluded that the risk GLOB1310aH to soil micro-organisms is acceptable in accordance with all intended uses.

zRMS comments:

The risk assessment was performed in accordance with the: Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC" (Sanco/10329/2002 rev 2 final 17 October 2002r.) on the basis of the worst-case application scenario.

The predicted environmental concentrations in soil (PECsoil) of the active substances, major metabolites and formulation were taken into account for the risk assessment.

Although studies on the effects on soil carbon transformation are not a data requirement any more according to Regulation (EU) No. 284/2013, the available data are summarized above.

For the assessment of risk to micro-organisms for formulation and metabolites the endpoints from studies presented in Appendix 2 were used. For active substances the EU agreed endpoints were used.

Conclusion:
Since no effects (> 25%) were seen at application rates far higher than the values of PECsoil for active substances, their metabolites and formulation it can be concluded that application of Glosset Ace (GLOB1310aH), according to the GAP, will not cause any detrimental effect to soil micro-organisms

9.10 Effects on non-target terrestrial plants (KCP 10.6)

9.10.1 Toxicity data

Effects on non-target terrestrial plants of GLOB1310aH were not evaluated as part of the EU assessment of Aclonifen and Flufenacet. New data submitted with this application are listed in 0 summarised in 0.

The selection of studies and endpoints for the risk assessment deviates from the results of the EU review process. Justifications are provided below.

Table 0-1: Endpoints and effect values relevant for the risk assessment for non-target terrestrial plants

Species	Substance	Exposure System	Results	Reference
Lactuca sativa.$_{d}$ [1)] *Brassica napus* $_{d}$ [2)] *Lolium perenne* $_{m}$ [3)] *Beta vulgaris* $_{d}$ [4)] *Rapahanus sativus* $_{d}$ [5)] *Solanum lycopersicum* $_{d}$ [6)] *Allium cepa* $_{m}$ [7)] *Cucumis sativus* $_{d}$ [8)] *Avena sativa* $_{m}$ [9)] *Hordeum vumgare* $_{m}$ [10)]	GLOB1310aH	21 d Seedling emergence	[1)] ER_{50} emergence >69.9 mL formulation/ha (most sensitive species, lettuce) [1)] **ER_{50} plant weight > 69.9 mL** formulation/ha (most senstive species, lettuce) [1)] ER_{50} plant height > 69.9 mL formulation/ha (most senstive species, lettuce) [5)] ER_{50} phytotoxicity = 374.7 mL formulation/ha (most senstive species, radish)	Friedemann A, 2021 (21 46 PSE 0001)
Lactuca sativa.$_{d}$ [1)] *Brassica napus* $_{d}$ [2)] *Lolium perenne* $_{m}$ [3)] *Beta vulgaris* $_{d}$ [4)] *Rapahanus sativus* $_{d}$ [5)] *Solanum lycopersicum* $_{d}$ [6)] *Allium cepa* $_{m}$ [7)] *Cucumis sativus* $_{d}$ [8)] *Avena sativa* $_{m}$ [9)] *Hordeum vumgare* $_{m}$ [10)]	GLOB1310aH	21 d Seedling emergence	**HC5 = 110 mL** formulation/ha	Calculated with online tool “mosaic” (https://mosaic.univ-lyon1.fr/ssd) with the data from study Friedemann A, 2021 (21 46 PSE 0001)
Brassica napus $_{d}$ [1)] *Cucumis sativus* $_{d}$ [2)] *Lactuca sativa.*$_{d}$ [3)] *Glycine max* $_{d}$ [4)] *Lolium perenne* $_{m}$ [5)] *Sorghum bicolor* $_{m}$ [6)] *Beta vulgaris* $_{d}$ [7)] *Linum usitatissimum* $_{m}$ [8)] *Allium cepa* $_{m}$ [9)] *Avena sativa* $_{m}$ [10)]	GLOB1310aH	21 d Vegetative vigour	[4)] **ER_{50} plant weight = 83.7 mL/ha** (most sensitive species, lettuce [1,2)] ER_{50} plant height > 223.4 mL/ha (most sensitive species, oilseed rape, cucumber) [4)] ER_{50} phytotoxicity = 129.1 mL/ha (most sensitive species, lettuce)	Friedemann A, 2021 (21 46 PVV 0001)
Brassica napus $_{d}$ [1)] *Cucumis sativus* $_{d}$ [2)] *Lactuca sativa.*$_{d}$ [3)] *Glycine max* $_{d}$ [4)] *Lolium perenne* $_{m}$ [5)] *Sorghum bicolor* $_{m}$ [6)] *Beta vulgaris* $_{d}$ [7)] *Linum usitatissimum* $_{m}$ [8)] *Allium cepa* $_{m}$ [9)] *Avena sativa* $_{m}$ [10)]	GLOB1310aH	21 d Vegetative vigour	**HC5 = 100 mL/ha**	Calculated with online tool “mosaic” (https://mosaic.univ-lyon1.fr/ssd) with the data from study Friedemann A, 2021 (21 46 PVV 0001)

m: monocotyledonous; d: dicotyledonous

9.10.1.1 Justification for new endpoints

As GLOB1310aH has not been evaluated during EU review, a new risk assessment for both seedling emergence and vegetative vigour is provided here and is considered adequate.

9.10.2 Risk assessment

9.10.2.1 Tier-1 risk assessment (based screening data)

Not relevant.

9.10.2.2 Tier-2 risk assessment (based on dose-response data)

The risk assessment is based on the "Guidance Document on Terrestrial Ecotoxicology", (SANCO/10329/2002 rev.2 final, 2002). It is restricted to off-field situations, as non-target plants are non-crop plants located outside the treated area.

Firstly, a risk assessment based on a deterministic approach is presented below. In addition, a refinement based on the calculated HC5 is presented as well.

Table 0-2: Assessment of the risk for non-target plants due to the use of GLOB1310aH in winter cereals (2L/ha)

Intended use	Winter and Spring cereals			
Product	GLOB1914H			
Application rate (mL/ha)	1 x 1500 mL formulation/ha			
MAF	1			
Test species	**ER_{50} (mL/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (mL/ha)**	**TER criterion: TER ≥ 5**
Lactuca sativa (seedling emergence)	69.9	2.77	41.55	**1.68**
Lactuca sativa (vegetative vigour)	83.7	2.77	41.55	**2.01**
Intended use	Winter and Spring cereals			
Product	GLOB1914H			
Application rate (mL/ha)	1 x 2000 mL formulation/ha			
MAF	1			
Test species	**ER_{50} (g/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (g/ha)**	**TER criterion: TER ≥ 5**
Lactuca sativa (seedling emergence)	69.9	2.77	55.4	**1.26**
Lactuca sativa (vegetative vigour)	83.7	2.77	55.4	**1.51**

9.10.2.3 Higher-tier risk assessment

As a refinement the calculated HC5 is used for the risk assessment. To achieve a concise risk assessment, the risk envelope approach is applied. Here, the assessment for the use 2L GLOB131aH/ha (the highest intended application rate) also covers the risk for non-target terrestrial plants from all other intended uses.

Table 0-3: Assessment of the risk for non-target plants due to the use of GLOB1310aH in winter cereals (2L/ha)

Intended use	Winter cereals			
Active substance/product	GLOB1310aH			
Application rate (L/ha)	1 x 2L/ha			
MAF	1			
Test species	**ER_{50} (mL/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (mL/ha)**	**TER criterion: TER ≥ 1**
HC5 seedling emergence	110	2.77%	55.4	1.99
HC5 vegetative vigour	100	2.77%	55.4	1.26

MAF: Multiple application factor; PER: Predicted environmental rate; TER: toxicity to exposure ratio. TER values shown in bold fall below the relevant trigger.

9.10.2.4 Risk mitigation measures

In order to reduce the off-field exposure, risk mitigation measures can be implemented. These correspond to unsprayed in-field buffer strips of a given width and/or the usage of drift reducing nozzles. The results of the risk assessment using typical mitigation measures (no-spray buffer zones of 5 or 10 m; drift-reducing nozzles with reduction by 50 %, 75 %, or 90 %) are summarised in the following table.

Table 0-4: Risk assessment for non-target terrestrial plants due to the use of GLOB1310aH in winter cereals (uses 1-6, 1.5L/ha) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use		Winter cereals			
Product		GLOB1310aH			
Application rate (mL/ha)		1 x 1500 mL formulation/ha			
MAF		1			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off\text{-}field}$ (mL/ha)**	**$PER_{off\text{-}field}$ 50 % drift red. (mL/ha)**	**$PER_{off\text{-}field}$ 75 % drift red. (mL/ha)**	**$PER_{off\text{-}field}$ 90 % drift red. (mL/ha)**
1	2.77	41.55	20.77	10.38	4.15
5	0.57	8.55	4.27	2.13	0.85
10	0.29	4.35	2.17	1.08	0.43
Toxicity value ER_{50} = 69.9 mL/ha Seedlng emergence		**TER criterion: TER ≥ 5**			
1		**1.68**	**3.36**	6.73	16.82
5		8.18	16.35	32.70	81.75

10	16.07	32.14	64.28	160.69

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in bold breach the relevant trigger.

Table 0-5: Risk assessment for non-target terrestrial plants due to the use of GLOB1310aH in winter cereals (uses 7-12, 2.0L/ha) considering risk mitigation (in-field no-spray buffer zones, and drift-reducing nozzles)

Intended use **Product** **Application rate (mL/ha)** **MAF**		Winter cereals GLOB1310aH 1 x 2000 mL formulation/ha 1			
Buffer strip (m)	**Drift rate (%)**	**$PER_{off-field}$ (mL/ha)**	**$PER_{off-field}$ 50 % drift red. (mL/ha)**	**$PER_{off-field}$ 75 % drift red. (mL/ha)**	**$PER_{off-field}$ 90 % drift red. (mL/ha)**
1	2.77	55.4	27.7	13.85	5.54
5	0.57	11.4	5.7	2.85	1.14
10	0.29	5.8	2.9	1.45	0.58
Toxicity value ER_{50} = 69.9 mL/ha Seedlng emergence		**TER** **criterion: TER ≥ 5**			
1		**1.26**	**2.52**	5.05	12.62
5		6.13	12.26	24.53	61.32
10		12.05	24.10	48.21	120.5

MAF: Multiple application factor; PER: Predicted environmental rates; TER: toxicity to exposure ratio. Criteria values shown in bold breach the relevant trigger.

For all intended uses of GLOB1310aH in winter cereals the calculations shows that the TER is higher than 5 when a buffer strip of 5 m is respected or with the use of 75% drift reduction. However, these mitigation measures doesn't have to be implemented because an undue risk to non-target plants was demonstrated when incorporated HC5 in the calculations in a refinement approach.

9.10.3 Overall conclusions

The risk to non-target terrestrial plants is considered acceptable when GLOB1310aH is used according to the proposed GAP.

zRMS Comments:

The risk assessment for non-target terrestrial plants was performed in accordance with the recommendations of the "Guidance Document on Terrestrial Ecotoxicology", as provided by the Commission Services (SANCO/10329/2002 rev 2 (final), October 17, 2002).

The risk assessment was based on the results of studies for formulation presented in Appendix 2 (vegetative vigour and on seedling emergence). Lettuce was the most sensitive species in both tests.

In the deterministic approach, the risk assessment was based on the most sensitive species. The TER

value is above the trigger value of 5 when 5 m buffer strip or 75% drift reduction is applied.

In order to conduct the probabilistic risk assessment the HC5 had to be derived from the dataset obtained in both studies. The HC5 calculations were conducted by online tool "MOSAIC$_{SSD}$" (https://mosaic.univ-lyon1.fr/ssd).

The EU guidance for terrestrial ecotoxicology states: "*Probabilistic methods that make use of the species sensitivity distribution would be straightforward in this assessment step as data from 6-10 species are available. Furthermore, a probabilistic approach is considered more suitable than the deterministic one to achieve the type of environmental goal mentioned above. This approach requires that log-normal or another defined type of distribution of the data has been shown to fit the data adequately. If the ED50 for less than 5 % of the species is below the highest predicted exposure level, the risk for terrestrial plants is assumed to be acceptable*".

Since 10 plant species have been tested, a probabilistic approach is justified.

The HC5 calculation was repeated by the zRMS, using MOSAIC$_{SSD}$ tool, as indicated by the applicant, because it is not clear which values were used.

Seedling emergence

ER50 values per species (mL product/ha)

	Seedling emergence	Plant survival	Plant height	Biomass reduction
Lettuce	> 69.9	> 69.9	> 69.9	> 69.9
Oilseed rape	> 214.1	> 214.1	> 214.1	> 214.1
Ryegrass	> 214.1	> 214.1	> 214.1	> 214.1
Sugar beet	> 374.7	> 374.7	> 374.7	> 374.7
Radishes	> 655.7	> 655.7	**632.9**	**551.5**
Tomato	> 655.7	> 655.7	> 655.7	> 655.7
Onion	> 655.7	> 655.7	> 655.7	> 655.7
Cucumber	> 655.7	> 655.7	> 655.7	> 655.7
Oat	> 1147.4	> 1147.4	> 1147.4	> 1147.4
Barley	> 2008.0	> 2008.0	> 2008.0	> 2008.0

The results presented below show that only two values are equal to-figure. There is no common agreement whether to exclude or to include greater than-figure values for the HR5-calculation. The exclusion of greater than-figures can be regarded as a very conservative approach. The ER50 levels obtained from the test contain only two values equal to-figures, therefore HC5 calculation was based on the all lowest ER50 values from each species. The greater than-figure values were included as equal to-figure.

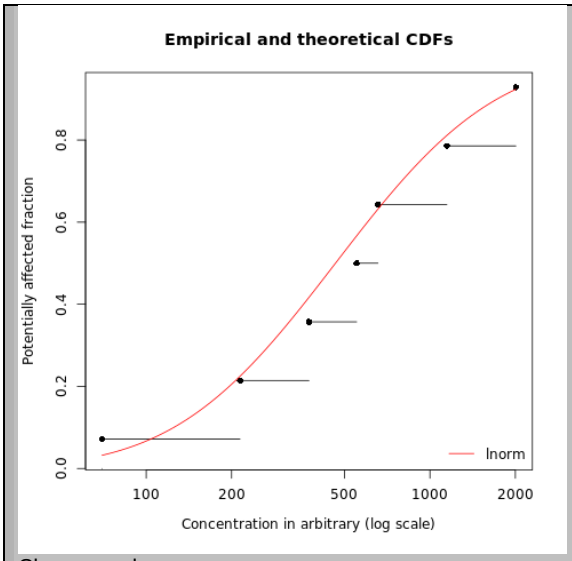
The SSD of are presented below:

MOSAIC*SSD*
Wed Jul 06 2022 | 13:11:40 GMT+0200 (czas środkowoeuropejski letni)

Dataset: Seedling emergence

- Estimated distribution
- Estimated hazardous concentration
- R script

Estimated distributions



Change scale

Log normal distribution (log-likelihood = -53.1)

meanlog: 6.1 [5.4 ; 6.9]

sdlog: 1 [0.42 ; 1.5]

Estimation of the hazardous concentration

HC	Log-normal
HC5	86 [32 ; 3.1e+02]
HC10	1.3e+02 [51 ; 3.9e+02]
HC20	2e+02 [88 ; 5.2e+02]
HC50	4.6e+02 [2.2e+02 ; 1e+03]

R script

This script may be used to reproduce the above analysis and can be modified to test other distributions.

```
# This script was generated by MOSAIC, a web application dedicated to
# ecotoxicology. It is available at http://pbil.univ-lyon1.fr/software/mosaic/

# To use this script, it is recommended to consult the reference manual of the
# fitdistrplus package http://cran.r-project.org/web/packages/fitdistrplus/fitdistrplus.pdf

# For any further question, please contact us at mosaic@univ-lyon1.fr

library(fitdistrplus)
library(actuar)

data <- c(69.9,214.1,374.7,551.5,655.7,1147.4,2008.)
distributions <- list('lnorm')


ft_lnorm <- fitdist(data,'lnorm')
summary(ft_lnorm)

cdfcomp(list(ft_lnorm),xlogscale=TRUE,legendtext = list('lnorm'),xlab='Concentration in log
scale',ylab='Potentially affected fraction')
```

```
bt_lnorm <- bootdist(ft_lnorm,niter=5000)
quantile(bt_lnorm,probs=c(0.05,0.1,0.2,0.5))
```

Vegetative vigour

ER50 values per species (mL product/ha)

	Plant survival	Plant height	Biomass reduction
Oilseed rape	> 223.4	> 223.4	> 223.4
Cucumber	> 223.4	> 223.4	> 223.4
Lettuce	**269.0**	**227.1**	**83.7**
Soybean	> 386.5	> 386.5	> 386.5
Ryegrass	> 386.5	> 386.5	> 386.5
Great millet	> 386.5	> 386.5	> 386.5
Sugar beet	> 1156.7	n.d.	**249.5**
Flax	**1149.0**	**459.7**	**213.9**
Onion	> 1156.7	> 1156.7	> 1156.7
Oat	> 1156.7	> 1156.7	**675.7**

The results presented above show that biomass reduction is the most sensitive, therefore these end-points were used for HC5 calculation. The ER50 levels obtained from the test contain four values equal to-figures.

Summary of ER50 used for HC5 calculation is presented below:

	Biomass reduction (ER50)	Comments	Endpoint included/excluded
Oilseed rape	> 223.4	rate-response presented and45.4% effect at 223.4 mL/ha	included as 223.4
Cucumber	> 223.4	no rate-response and 24.7% effect at 223.4 mL/ha	excluded
Lettuce	83.7		included
Soybean	> 386.5	no rate response and 21.5% effect at 386.5 mL/ha	excluded
Ryegrass	> 386.5	rate-response presented and46% effect at 386.5 mL/ha	included as 386.5
Great millet	> 386.5	no rate-response and -1.4% effect at 386.5 mL/ha	excluded
Sugar beet	249.5		included
Flax	213.9		included
Onion	> 1156.7	no rate-response and 18.2% effect at 1156.7 mL/ha	excluded
Oat	675.7		included

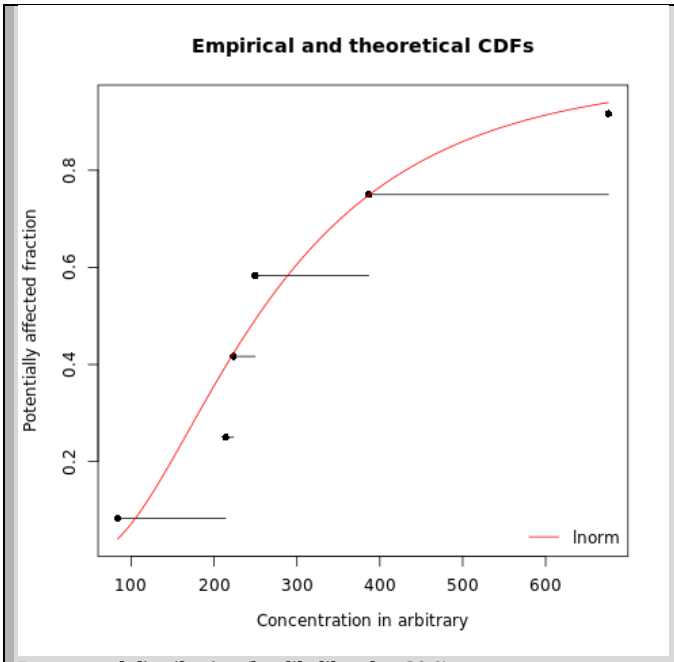
The SSD of are presented below:

MOSAIC$_{SSD}$
Wed Jul 06 2022 | 13:11:40 GMT+0200 (czas środkowoeuropejski letni)

Dataset: Vegetative vigour

Estimated distribution
Estimated hazardous concentration
R script

Estimated distributions



Log normal distribution (log-likelihood = -39.0)
meanlog: 5.5 [5 ; 6]
sdlog: 0.63 [0.24 ; 0.93]

Estimation of the hazardous concentration

HC	Log-normal
HC5	89 [47 ; 2.2e+02]
HC10	1.1e+02 [63 ; 2.5e+02]
HC20	1.5e+02 [89 ; 2.9e+02]
HC50	2.5e+02 [1.5e+02 ; 4.2e+02]

R script

This script may be used to reproduce the above analysis and can be modified to test other distributions.

```
# This script was generated by MOSAIC, a web application dedicated to
# ecotoxicology. It is available at http://pbil.univ-lyon1.fr/software/mosaic/

# To use this script, it is recommended to consult the reference manual of the
# fitdistrplus package http://cran.r-project.org/web/packages/fitdistrplus/fitdistrplus.pdf

# For any further question, please contact us at mosaic@univ-lyon1.fr

library(fitdistrplus)
library(actuar)

data <- c(223.4,83.7,386.5,249.5,213.9,675.7)
distributions <- list('lnorm')


ft_lnorm <- fitdist(data,'lnorm')
summary(ft_lnorm)

cdfcomp(list(ft_lnorm),xlogscale=TRUE,legendtext = list('lnorm'),xlab='Concentration in log
scale',ylab='Potentially affected fraction')

bt_lnorm <- bootdist(ft_lnorm,niter=5000)
quantile(bt_lnorm,probs=c(0.05,0.1,0.2,0.5
```

As the recalculated HC5 values are lower than those indicated by the applicants, therefore assessment of the risk for non-target terrestrial plants, using HC5 for seedling emergence and vegetative vigour is presented below:

Intended use	Winter cereals
Active substance/product	GLOB1310aH
Application rate (L/ha)	1 x 2L/ha
MAF	1

Test species	**ER_{50} (mL/ha)**	**Drift rate**	**$PER_{off\text{-}field}$ (mL/ha)**	**TER criterion: TER ≥ 1**
HC5 seedling emergence	86	2.77%	55.4	1.6
HC5 vegetative vigour	89	2.77%	55.4	1.6

Conclusion:
The risk for the terrestrial plants following use of GLOB1310aH/Glosset Ace could be considered as low when:

- taking into account the deterministic approach, when 5 m buffer strip or 75% drift reduction is applied;
- taking into account the probabilistic approach, without risk mitigation measured.

It should be mentioned that Member States should consider at national level which approach to risk assessment is acceptable and, in the case of a probabilistic approach, whether an assessment factor of 1 is sufficiently protective for non-target terrestrial plants.

9.11 Effects on other terrestrial organisms (flora and fauna) (KCP 10.7)

Tests on other non-target species are not required.

9.12 Monitoring data (KCP 10.8)

Not relevant.

9.13 Classification and Labelling

Based on the acute aquatic toxicity studies on Daphnia, algae and aquatic macrophytes (Lemna and Myriophyllum), GLOB1310aH must be classified as Acute Category 1 (H400) “Very toxic to aquatic life”, because ErC50 is below 1 mg/L for the aforementioned aquatic organisms.

For chronic classification, the summation method in accordance with EU Regulation 1272/2008 (CLP labelling) was applied. Please refer to Part C for detail on summation method. Following this method, it has been concluded that GLOB1310aH has to be classified as Chronic Cat. 1 (H410) “Very toxic to aquatic life with long lasting effects”.

Pictogram: GHS09
Signal word: Warning

H-statements

H400 Very toxic to aquatic life.
H410 Very toxic to aquatic life with long lasting effects.

P-statements

P391 Collect spillage.
P501 Dispose of contents/container to … in accordance with local/regional/national/international regulations (to be specified).

Other safety/precautionary phrases:

SP1: Do not contaminate water with the product or its container.
SPe3:
To protect aquatic organisms respect the following restrictions:

Country	1.5L/ha		2.0L/ha	
	Landscape Mitigation Report (Step4)	**VFSMOD**	**Landscape Mitigation Report (Step4)**	**VFSMOD**
Poland (D3, D4, R1)	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 10m vfs + 50% drn	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 10m vfs	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 20m vfs	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 10m vfs + 50% drn
Belgium (D3, D4, R1)	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 10m vfs + 50% drn	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 10m vfs	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 20m vfs	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 10m vfs + 50% drn
Hungary (D3, D5, R1, R3, R4)	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 20m vfs	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 20m vfs	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 20m vfs + 50% drn	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 20m vfs + 50% drn
Ireland (D4)	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb	-	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn	-
Romania (D4, D5, R1, R3, R4)	5m nsb + 75% drn OR 10m nsb + 50% drn OR	5m nsb + 75% drn OR 10m nsb + 50% drn OR	5m nsb + 90% drn OR 10m nsb + 75% drn OR	5m nsb + 90% drn OR 10m nsb + 75% drn OR

	20m nsb In run-off susceptible soils: 20m vfs	20m nsb In run-off susceptible soils: 20m vfs	15m nsb + 50% drn In run-off susceptible soils: 20m vfs + 50% drn	15m nsb + 50% drn In run-off susceptible soils: 20m vfs + 50% drn
Slovakia (D4, D5, R1)	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 10m vfs + 50% drn	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb In run-off susceptible soils: 10m vfs	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 20m vfs	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn In run-off susceptible soils: 10m vfs + 50% drn
Slovenia (only drift, run-off is not relevant)	5m nsb + 75% drn OR 10m nsb + 50% drn OR 20m nsb	-	5m nsb + 90% drn OR 10m nsb + 75% drn OR 15m nsb + 50% drn	-

EUH401 To avoid risks to human health and the environment, comply with the instructions for use.

Appendix 1 Lists of data considered in support of the evaluation

Tables considered not relevant can be deleted as appropriate.

MS to blacken authors of vertebrate studies in the version made available to third parties/public.

List of data submitted by the applicant and relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.2.1	Juckeland, D.	2021a	Acute toxicity of GLOB1310aH to Daphnia magna in a 48-hour static test Study No. 21 48 ADL 0001 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 10.2.1	Juckeland, D.	2021b	Effects of GLOB1310aH on *Lemna gibba* in a growth inhibition test under semi-static test conditions Study No. 21 48 ALE 0001 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 10.2.1	Juckeland, D.	2021c	Effects of GLOB1310aH on Myriophyllum spicatum in a semi-static water-sediment system Study No. 21 48 AMS 0001 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 10.2.1	Juckeland, D.	2021d	Effects of GLOB1310aH on *Desmodesmus subspicatus* in an algal growth inhibition test Study No. 21 48 AAL 0017 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
KCP 10.2.1	Juckeland, D.	2021e	Effects of GLOB1310aH on *Pseudokirchneriella subcapitata* in an algal growth inhibition test Study No. 21 48 AAL 0017 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 10.3.1.1.1 & KCP 10.3.1.1.2	Franke, M.	2021	Acute toxicity of GLOB1310aH to the honeybee *Apis mellifera* L. under laboratory conditions Study No. 21 48 BAA 0001 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 10.3.1.1.2	Amsel, K.	2021	Acute toxicity of GLOB1310aH to the bumblebee *Bombus terrestris* L. under laboratory conditions Study No. 21 48 BBA 0001 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 10.3.1.2	Dressler, K.	2021	Chronic toxicity of GLOB1310aH to the honey bee *Apis mellifera* L. under laboratory conditions Study No. 21 48 BAC 0004 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 10.3.1.3	Hänsel, M.	2021	GLOB1310aH - Repeated exposure of honey bee (*Apis mellifera* L.) larvae under laboratory conditions Study No. 21 48 BLC 0003 Biochem Agrar GmbH, Germany GLP Unpublished		
KCP 10.3.2	Röhlig, U.	2021a	Effects of GLOB1310aH on the carabid beetle *Poecilus cupreus* L. in an extended laboratory test (Limit-test). Study No. 21 48 NLE 0001 Biochem Agrar GmbH, Germany GLP	N	Globachem NV

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Unpublished		
KCP 10.3.2	Röhlig, U.	2021b	Effects of GLOB1310aH on the rove beetle *Aleochara bilineata* GYLL. in an extended laboratory test (Limit-test) Study No. 21 48 NKE 0001 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 10.3.2	Röhlig, U.	2021c	Effects of GLOB1310aH on the parasitic wasp *Aphidius rhopalosiphi* (DESTEFANI-PEREZ) in an extended laboratory test Study No. 21 48 NAE 0007 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 10.3.2	Röhlig, U.	2021d	Effects of GLOB1310aH on the parasitic wasp *Aphidius rhopalosiphi* DESTEFANI-PEREZ in an extended laboratory test (under semi-field conditions aged residues on potted bean plants) Study No. 21 48 NAR 0001 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 10.3.2	Röhlig, U.	2021f	Effects of GLOB1310aH on the predatory mite *Typhlodromus pyri* SCHEUTEN in an extended laboratory test Study No. 21 48 NTE 0008 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 10.3.2	Röhlig, U.	2021g	Effects of GLOB1310aH on the predatory mite *Typhlodromus pyri* SCHEUTEN in an extended laboratory test (under semi-field conditions aged residues on bean plants)Study No. 21 48 NTR 0001	N	Globachem NV

Data point	Author(s)	Year	**Title** **Company Report No.** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Vertebrate study Y/N**	**Owner**
			Biochem Agrar GmbH, Germany GLP Unpublished		
KCP 10.4.1	Friedrich, S.	2021a	Effects of GLOB1310aH on the reproduction of the earthworm *Eisenia fetida* in artificial soil Study No. 21 48 TEC 0003 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 10.4.2	Friedrich, S.	2021b	Effects of GLOB1310aH on the reproduction of the collembolan *Folsomia candida*. Study No. 21 48 TCC 0002 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 10.4.2	Schulz, L.	2021a	Effects of GLOB1310aH on the reproduction of the predatory mite *Hypoaspis aculeifer*. Study No. 21 48 THC 0001 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 10.5	Schulz, L.	2021b	Effects of GLOB1310aH on the activity of soil microflora (Nitrogen transformation test) Study No. 21 48 SMN 0001 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 10.5	Servajean	2014	Soil micro-organisms: Nitrogen and carbon transformation test with FOE sulfonic acid (OECD 216 and OECD 217, January 2000) Report No. 14-99-006-ES Phytosafe GLP Unpublished	N	Globachem NV

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP 10.5	Servajean	2014	Soil micro-organisms: Nitrogen and carbon transformation test with FOE oxalate (OECD 216 and OECD 217, January 2000) Report No. 14-99-007-ES Phytosafe GLP Unpublished	N	Globachem NV
KCP 10.6.2	Friedemann, A.	2021a	Effects of GLOB1310aH on vegetative vigour of ten non-target terrestrial plant species under greenhouse conditions. Study No. 21 46 PVV 0001 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV
KCP 10.6.2	Friedemann, A.	2021b	Effects of GLOB1310aH on seedling emergence and seedling growth of ten non-target terrestrial plant species under greenhouse conditions. Study No. 21 46 PSE 0001 Biochem Agrar GmbH, Germany GLP Unpublished	N	Globachem NV

List of data submitted or referred to by the applicant and relied on, but already evaluated at EU peer review

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP XX	Author	YYYY	Title Company Report N	Y/N	Owner

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
			Source GLP/non GLP/GEP/non GEP Published/Unpublished		

The following tables are to be completed by MS

List of data submitted by the applicant and not relied on

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

List of data relied on not submitted by the applicant but necessary for evaluation

Data point	Author(s)	Year	Title Company Report No. Source (where different from company) GLP or GEP status Published or not	Vertebrate study Y/N	Owner
KCP XX	Author	YYYY	Title Company Report N Source GLP/non GLP/GEP/non GEP Published/Unpublished	Y/N	Owner

Appendix 2 Detailed evaluation of the new studies

A 2.1 KCP 10.1 Effects on birds and other terrestrial vertebrates

A 2.1.1 KCP 10.1.1 Effects on birds

No new studies on birds are submitted within this application.

A 2.1.2 KCP 10.1.2 Effects on terrestrial vertebrates other than birds

No new studies on birds are submitted within this application.

A 2.1.3 KCP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)

No new studies on other terrestrial vertebrate wildlife are submitted within this application.

A 2.2 KCP 10.2 Effects on aquatic organisms

A 2.2.1 KCP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

Comments of zRMS:	The study was performed according to OECD TG 202 and principles of GLP. The validity criteria are met. The immobility in the control group is below 10% (observed 0%) The measured, lowest value of oxygen concentration at the end of the test is at 7,84 mg/L (required ≥ 3 mg/L). The study is considered acceptable and suitable for the risk assessment. All results refer to nominal concentrations since the measured concentrations were within 80 to 120% of nominal.

Reference:	KCP 10.2.1
Report	Acute toxicity of GLOB1310aH to *Daphnia magna* in a 48-hour static test. Juckeland, D., 2021a.Study No. 21 48 ADL 0001
Guideline(s):	Yes ~~(OECD 213~~ (1998, OECD 202 (2004)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

SUMMARY

Study title:	Acute toxicity of GLOB1310aH to *Daphnia magna* in a 48-hour static test
Guideline:	OECD 202 (2004)
BioChem project No.:	21 48 ADL 0001
Test item:	GLOB1310aH Batch no.: GLO-20F-2306A Content of active substances (analysed) Aclonifen: 543.7 g/L Flufenacet: 61.55 g/L
Test species:	*Daphnia magna* STRAUS
Test system:	Exposure of *Daphnia* to the test item applied in test medium (dilution water)

Test conditions

Temperature	20.4 – 20.5 °C
Photoperiod:	16 h light : 8 h dark, daily (approximately 20 µE m^{-2} s^{-1}
Treatments:	Control (untreated test medium) test item (GLOB1310aH)
Number of test vessels/concentration:	4
Number of *Daphnia*/concentration:	20
Test concentration (nominal):	2.39, 3.82, 6.10, 9.78, 15.6, 25.0 mg/L test item equivalent to 1.06, 1.69, 2.70, 4.33, 6.92, 11.1 mg/L Aclonifen 0.120, 0.191, 0.305, 0.490, 0.784, 1.25 mg/L Flufenacet
Exposure time:	48 hours (static test procedure)
Biological observations:	Number of immobilised *Daphnia*: after 3, 24 and 48 hours
Statistics:	Fisher`s Exact Binomial Test with Bonferroni Correction and Step-down Cochran-Armitage Test Procedure for statistical significance of immobility (alpha = 0.05, one-sided) Probit analysis for calculation of the EC_x for immobility (with 95% confidence limits) Statistical program: ToxRat Professional Version 3.3; 20.10.2018 (RATTE)
Dates of work:	
Biological phase:	experimental start date: 05.05.2021 experimental completion date: 07.05.2021
Analytical phase:	experimental start: 26.05.2021 experimental completion: 27.05.2021

Findings

The measured concentrations of Aclonifen were within ranges of 103.4 – 119.3% of nominal concentrations in the freshly prepared test solutions at the start of the test and within a range of 95.6 – 114.0% in the spent solutions at the test end (48 hours) based on nominal values.

Measured concentrations of Flufenacet in test solutions were within ranges of 104.4 – 116.9% of nominal concentrations in the freshly prepared test solutions at the start of the test and within a range of 105.6 – 119.3% in the spent solutions at the test end (48 hours) based on nominal values.

Therefore, the calculated endpoints are based on the nominal concentrations for the test item and on the active substances, since the measured concentrations were within 80 to 120% of nominal.

Table 3: Effects of the test item on immobility of *Daphnia magna*

Effect concentration	**GLOB1310aH, mg/L nominal**					
	24 h			**48 h**		
	after application					
NOEC						
test item	**≥ 25.0**			**2.39**		
Aclonifen	**≥ 11.1**			**1.06**		
Flufenacet	**≥ 1.25**			**0.120**		
LOEC						
test item	**> 25.0**			**3.82**		
Aclonifen	**> 11.1**			**1.69**		
Flufenacet	**> 1.25**			**0.191**		
EC and 95% CI (lower – upper)	**EC_{10}**	**EC_{20}**	**EC_{50}**	**EC_{10}**	**EC_{20}**	**EC_{50}**
test item	**15.0** (8.27 – 19.8)	**20.1** (14.8 – 32.6)	**34.9** (24.7 – 154.2)	**2.90** (1.97 – 3.67)	**3.74** (2.77 – 4.57)	**6.12** (5.08 – 7.31)
Aclonifen	**6.64** (3.66 – 8.78)	**8.88** (6.56 – 14.4)	**15.5** (10.6 – 68.2)	**1.28** (0.872 – 1.62)	**1.66** (1.23 – 2.02)	**2.71** (2.25 – 3.24)
Flufenacet	**0.752** (0.414 – 0.994)	**1.01** (0.742 – 1.63)	**1.75** (1.24 – 7.73)	**0.145** (0.099 – 0.184)	**0.187** (0.139 – 0.229)	**0.307** (0.255 – 0.366)

Calculations were conducted using unrounded values

Table 4: Observations

Time after application	Test concentration mg/L test item nominal						
	Control	**2.39**	**3.82**	**6.10**	**9.78**	**15.6**	**25.0**
	Test concentration mg/L Aclonifen, nominal						
	Control	**1.06**	**1.69**	**2.70**	**4.33**	**6.92**	**11.1**
	Test concentration mg/L Flufenacet, nominal						
	Control	**0.120**	**0.191**	**0.305**	**0.490**	**0.784**	**1.25**
	Immobility (%)						
3 h	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24 h	0.0	0.0	0.0	0.0	0.0	20.0	25.0
48 h	0.0	0.0	30.0 +	55.0 +	70.0 +	95.0 +	100.0 +

* significantly different from the control
(Step-down Cochran-Armitage Test, alpha = 0.05, one-sided greater)

Conclusion:

An acute immobilisation test was performed to assess the effects of the test item GLOB1310aH on *Daphnia magna* during 48 hours of static exposure.

Significant effects on immobility were found using Step-down Cochran-Armitage Test (alpha = 0.050, one-sided greater) at the nominal test concentrations ≥ 3.82 mg/L test item at 48 hours.

As a result, a LOEC of 3.82 mg/L test item (equivalent to 1.69 mg/L Aclonifen and 0.191 mg/L Flufenacet, nominal) was determined. The corresponding NOEC was 2.39 mg/L test item, nominal (equivalent to 1.06 mg/L Aclonifen and 0.120 µg/L Flufenacet, nominal). The EC_{50} for immobility was 6.12 mg/L test item (equivalent to 2.71 mg/L Aclonifen and 0.307 mg/L Flufenacet, nominal) at 48 hours.

Comments of zRMS:	The study was performed according to OECD TG 221 and principles of GLP. The validity criteria are met. The doubling time of frond numbers in the control was less than 2.5 days (actually 2.18 days), as required by OECD 221 Guideline. The study is considered acceptable and suitable for the risk assessment. The calculated study endpoints are based on the mean measured concentrations for the test substances Aclonifen and Flufenacet as well as test item, recalculated, because the recoveries for these active substances were not between 80 and 120% of nominal.

Reference:	KCP 10.2.1
Report	Effects of GLOB1310aH on *Lemna gibba* in a growth inhibition test under semi-static test conditions. Juckeland, D., 2021b. Study No. 21 48 ALE 0001
Guideline(s):	Yes (OECD 221 (2006) ~~213 (1998, OECD 202 (2004)~~
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

SUMMARY

Study title:	Effects of GLOB1310aH on *Lemna gibba* in a growth inhibition test under semi-static test conditions
Guideline:	OECD 221 (2006)
BioChem project No.:	21 48 ALE 0001
Test item:	GLOB1310aH Batch no.: GLO-20F-2306A Content of active substances (analysed) Aclonifen: 543.7 g/L Flufenacet: 61.55 g/L
Test species:	Duckweed – *Lemna gibba* L.
Test system:	Exposure of *Lemna gibba* to the test item applied in test medium (semi-static conditions)

Test conditions

Temperature:	22.9 – 23.1°C (recorded in the water bath)
Lighting:	continuous illumination (on average 127 µE x m^{-2} x s^{-1})
Treatments:	control, untreated test medium, test item (GLOB1310aH)
Test concentration (nominal)[1]:	0.41, 1.23, 3.70, 11.1, 33.3, 100.0 µg/L test item equivalent to 0.18, 0.55, 1.64, 4.92, 14.8, 40.5 µg/L Aclonifen 0.02, 0.07, 0.21, 0.59, 1.72, 5.38 µg/L Flufenacet
Test concentrations (mean measured a.s.):	0.14, 0.49, 1.31, 3.83, 12.0, 40.5 µg/L Aclonifen 0.023, 0.068, 0.209, 0.586, 1.72, 5.38 µg/L Flufenacet 0.33, 1.12, 3.09, 8.96, 27.8, 93.1. µg/L test item, recalculated
Exposure time:	7 days (semi-static test procedure)
Biological observations:	Frond number: day 0, 2, 5 and 7 Changes in plant development: day 0, 2, 5 and 7 Dry weight: day 0 and 7
Statistics:	LOEC/NOEC: Williams t-test, Welch-t-test; (alpha = 0.05, one-sided smaller) EC_x-values: linear regression: Probit analysis using linear maximum likelihood regression ToxRat Professional Version 3.3 (20.10.2018)
Dates of work:	
Biological phase:	experimental start date: 10.05.2021 experimental completion date (determination dry weight) : 03.06.2021
Analytical phase:	experimental start: 16.06.2021 experimental completion date: 29.06.2021

* nominal test concentrations based on the weighed amount of test item (mean values of day 0, day 2 and day 5)
TW mean – time weighted mean

Findings:
The measured concentrations of Aclonifen remained within a range of 51.6 – 129.7% of nominal concentrations in the freshly prepared test solutions at test start and at each renewal in the freshly prepared test solutions. The Aclonifen concentrations in the spent test solutions were determined at 65.4 – 97.0% of nominal at each renewal and at the end of the test (day 7).

The measured concentrations of Flufenacet remained within a range of 98.7 – 147.3% of nominal concentrations in the freshly prepared test solutions at test start and at each renewal in the freshly prepared test solutions. The Flufenacet concentrations in the spent test solutions were determined at 98.5 – 118.4% of nominal at each renewal and at the end of the test (day 7).

Therefore, the calculated study endpoints are based on nominal and on the mean measured concentrations for the test substances Aclonifen and Flufenacet as well as test item, recalculated, because the recoveries for these active substances were not between 80 and 120% of nominal.

Table 5**:** LOEC, NOEC and effect concentrations EC_x of GLOB1310aH for growth rate and yield based on frond number and biomass for *Lemna gibba* at day 7 d

Effect concentration	**GLOB1310aH, µg/L**			
	average specific growth rate inhibition		yield inhibition	
	Frond number	Biomass	Frond number	Biomass
NOEC				
test item, nominal	**0.41**	**0.41**	**0.41**	**0.41**
Aclonifen, nominal	**0.18**	**0.18**	**0.18**	**0.18**
Flufenacet, nominal	**0.021**	**0.021**	**0.021**	**0.021**
Aclonifen, mean measured	**0.14**	**0.14**	**0.14**	**0.14**
Flufenacet, mean measured	**0.023**	**0.023**	**0.023**	**0.023**
test item, mean measured recalculated	**0.33**	**0.33**	**0.33**	**0.33**
LOEC				
test item, nominal	**1.23**	**1.23**	**1.23**	**1.23**
Aclonifen, nominal	**0.55**	**0.55**	**0.55**	**0.55**
Flufenacet, nominal	**0.062**	**0.062**	**0.062**	**0.062**
Aclonifen, mean measured	**0.48**	**0.48**	**0.48**	**0.48**
Flufenacet, mean measured	**0.068**	**0.068**	**0.068**	**0.068**
test item, mean measured recalculated	**1.12**	**1.12**	**1.12**	**1.12**

CI - confidence interval
Calculations performed using unrounded values

Table 1**:** LOEC, NOEC and effect concentrations EC_x of GLOB1310aH for growth rate and yield based on frond number and biomass for *Lemna gibba* at day 7 d (continued)

Effect concentration	**GLOB1310aH, µg/L**			
	average specific growth rate inhibition		yield inhibition	
	Frond number	Biomass	Frond number	Biomass
EC_{10} (CI, lower - upper)	**E_rC_{10}**	**E_rC_{10}**	**E_yC_{10}**	**E_yC_{10}**
test item, nominal	**2.48** (1.92 – 3.06)	**1.35** (0.74 – 2.07)	**1.10** (0.91 – 1.30)	**0.83** (0.62 – 1.05)
Aclonifen, nominal	**1.10** (0.85 – 1.35)	**0.60** (0.33 – 0.92)	**0.49** (0.40 – 0.58)	**0.37** (0.27 – 0.46)
Flufenacet, nominal	**0.12** (0.10 – 0.15)	**0.068** (0.037 – 0.10)	**0.055** (0.046 – 0.065)	**0.042** (0.031 - 0.053)
Aclonifen, mean measured	**0.86** (0.67 – 1.05)	**0.47** (0.25 – 0.74)	**0.41** (0.34 – 0.48)	**0.32** (0.23 – 0.41)
Flufenacet, mean measured	**0.14** (0.11 – 0.17)	**0.075** (0.040 – 0.12)	**0.063** (0.053 – 0.074)	**0.048** (0.036 - 0.060)
test item, mean measured recalculated	**2.02** (1.58 – 2.47)	**1.11** (0.58 – 1.74)	**0.96** (0.80 – 1.12)	**0.74** (0.55 – 0.95)
EC_{20} (CI, lower - upper)	**E_rC_{20}**	**E_rC_{20}**	**E_yC_{20}**	**E_yC_{20}**
test item, nominal	**4.50** (3.72 – 5.27)	**3.00** (1.94 – 4.15)	**2.01** (1.74 – 2.28)	**1.47** (1.18 – 1.75)
Aclonifen, nominal	**1.99** (1.65 – 2.33)	**1.33** (0.86 – 1.84)	**0.89** (0.77 – 1.01)	**0.65** (0.52 – 0.77)
Flufenacet, nominal	**0.23** (0.19 – 0.26)	**0.15** (0.10 – 0.21)	**0.10** (0.087 – 0.11)	**0.074** (0.059 – 0.09)
Aclonifen, mean measured	**1.57** (1.31 – 1.83)	**1.06** (0.66 – 1.49)	**0.73** (0.64 – 0.83)	**0.55** (0.44 – 0.66)
Flufenacet, mean measured	**0.25** (0.21 – 0.29)	**0.16** (0.11 – 0.23)	**0.11** (0.099 – 0.13)	**0.083** (0.067 - 0.098)
test item, mean measured recalculated	**3.68** (3.07 – 4.29)	**2.49** (1.55 – 3.49)	**1.72** (1.51 – 1.93)	**1.29** (1.02 – 1.54)

CI - confidence interval
Calculations performed using unrounded values

Table 1**:** LOEC, NOEC and effect concentrations EC_x of GLOB1310aH for growth rate and yield based on frond number and biomass for *Lemna gibba* at day 7 d (continued)

Effect concentration	**GLOB1310aH, µg/L**			
	average specific growth rate inhibition		yield inhibition	
	Frond number	Biomass	Frond number	Biomass
EC_{50} (CI, lower - upper)	**E_rC_{50}**	**E_rC_{50}**	**E_yC_{50}**	**E_yC_{50}**
test item, nominal	**14.0** (12.6 – 15.7)	**13.9** (10.9 – 17.6)	**6.35** (5.82 – 6.92)	**4.32** (3.80 – 4.90)
Aclonifen, nominal	**6.21** (5.56 – 6.94)	**6.13** (4.84 – 7.81)	**2.81** (2.58 – 3.06)	**1.91** (1.68 – 2.17)
Flufenacet, nominal	**0.70** (0.63 – 0.79)	**0.69** (0.55 – 0.88)	**0.32** (0.29 – 0.35)	**0.22** (0.19 – 0.25)
Aclonifen, mean measured	**4.96** (4.45 – 5.54)	**4.97** (3.84 – 6.49)	**2.26** (2.08 – 2.45)	**1.55** (1.36 – 1.76)
Flufenacet, mean measured	**0.74** (0.67 – 0.83)	**0.74** (0.58 – 0.94)	**0.35** (0.32 – 0.37)	**0.24** (0.21 – 0.27)
test item, mean measured recalculated	**11.6** (10.4 – 12.9)	**11.6** (8.97 – 15.1)	**5.28** (4.87 – 5.72)	**3.62** (3.18 – 4.12)

CI - confidence interval
Calculations performed using unrounded values

Table 6: Effects of GLOB1310aH on growth rate and yield for *Lemna gibba*

Treatment group µg/L test item nominal	**Final frond number** replicate mean day 7	**Biomass (dry weight)** replicate mean day 7 (mg)	**% Inhibition**			
			Average specific growth rate (% I_r)		**yield (% I_y)**	
			frond number	biomass	frond number	biomass
Control	71.0	8.5	n.r.	n.r.	n.r.	n.r.
0.41	72.7	8.3	-1.1	0.8	-2.7	2.3
1.23	64.0	7.5	5.0 +	4.8 +	11.3 +	12.9 +
3.70	48.0	4.6	19.0 +	23.1 +	37.1 +	49.3 +
11.1	32.3	2.5	38.1 +	45.8 +	62.4 +	75.5 +
33.3	14.0	1.1	78.6 +	77.4 +	91.9 +	93.7 +
100.0	11.0	1.2	90.4 +	74.9 +	96.8 +	92.8 +

n.r. – not relevant
* negative values mean a higher growth compared to the control
+ significantly different to the control (Williams t-test; Welch-t-test; alpha = 0.05, one-sided)

Validity criteria

The test was valid because the doubling time of frond numbers in the control was less than 2.5 days (actually 2.18 days), as required by OECD 221 Guideline (2006).

The validity criterion was accomplished as follows:

According to the guideline, the doubling time of the frond number in the control must be less than 2.5 d (60 h), corresponding to approximately a 7-fold increase in biomass in 7 days and an average specific growth rate of 0.275 d^{-1}. The measured doubling time of the frond numbers in the control was on average 2.35 days (1.81 days for dry weight), corresponding to a 7.9-fold increase in frond number over the 7-day study period (mean of 9 to 71.0 fronds in the control vessels) and a 14.2-fold increase in dry weight (0.60 mg to 8.5 mg dry weight). The average specific growth rate in the control was 0.295 d^{-1} for frond number and 0.378 d^{-1} for dry weight.

The E_rC_{50} (growth rate based on frond number) value for the reference item (toxic standard) 3,5-dichlorophenol was 3.27 mg/L. This value is included in the range 2.2 - 3.8 mg/L 3,5-dichlorophenol as stated in Guideline ISO 20079, demonstrating that the test system was sensitive.

Conclusions:

A *Lemna* growth inhibition test was performed to assess the effects of the test item GLOB1310aH (active substances: Aclonifen and Flufenacet) to *Lemna gibba* (duckweed) during 7 days of exposure in a semi-static test design.

No statistically significant effect on yield and growth rate of *Lemna* based on frond number and biomass was observed at the nominal concentration of 0.14 µg/L test item, whereas statistically significant effects (alpha = 0.05) were calculated for nominal concentrations ≥ 1.23 µg/L test item. As a result, the NOEC for yield and growth rate based on frond number and biomass was determined to be 0.41 µg/L test item (equivalent to 0.14 µg/L Aclonifen and 0.023 µg/L Flufenacet, mean measured) and the LOEC was determined to be 304.7 µg/L test item (equivalent to 0.48 µg/L Aclonifen and 0.068 µg/L Flufenacet, mean measured), based on nominal concentrations.

Based on nominal concentrations the lowest EC_{50}-value (0-7 d) was 4.32 µg/L test item (equivalent to 3.62 µg/L test item, mean measured recalculated) for yield based on biomass.

Based on nominal concentrations the EC_{50}-value (0-7 d) was 14.0 µg/L test item (equivalent to 11.6 µg/L test item, mean measured recalculated) for growth rate based on frond number.

Based on nominal concentrations the EC_{50}-value (0-7 d) was 6.35 µg/L test item (equivalent to 5.28 µg/L test item, mean measured recalculated) for yield based on frond number.

Based on nominal concentrations the EC_{50}-value (0-7 d) was 13.9 µg/L test item (equivalent to 11.6 µg/L test item, mean measured recalculated) for growth rate based on biomass.

Comments of zRMS:	The study was performed according to OECD TG 239 and principles of GLP. The validity criteria are met. The doubling of total shoot length and fresh weight in control plants: required factor: 2, achieved: factor 5.0 for total shoot length and factor 7.0 for fresh weight. Control plants did not show any visual symptoms of chlorosis and were visibly free from contamination by other organisms such as algae and/or bacterial films on the plants. No observations of the latter were made at the surface of the sediment and in test medium. The mean coefficient of variation for yield, based on measurements of shoot fresh weight in control cultures, does not exceed 35% (achieved: 7.2%), as required by OECD 221 Guideline. The study is considered acceptable and suitable for the risk assessment. The study endpoints are based on nominal and on the mean measured concentrations for the active substance Aclonifen as well as on the test item, recalculated, because the

	recoveries for Aclonifen were not between 80 and 120% of nominal.

Reference:	KCP 10.2.1
Report	Effects of GLOB1310aH on Myriophyllum spicatum in a semi-static water-sediment system. Juckeland, D., 2021c. Study No. 21 48 AMS 0001
Guideline(s):	Yes (OECD 239 (2014))
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

SUMMARY

Study title:	Effects of GLOB1310aH on *Myriophyllum spicatum* in a semi-static water-sediment system
Guideline:	OECD 239 (September 2014)
BioChem project No.:	21 48 AMS 0001
Test item:	GLOB1310aH Batch no.: GLO-20F-2306A Content of active substances (analysed) Aclonifen: 543.7 g/L Flufenacet: 61.55 g/L
Test species:	*Myriophyllum spicatum* L.
Test system:	exposure of *Myriophyllum spicatum* to the test item applied in test medium (semi-static conditions), no vehicle was used
Test conditions:	19.5 – 20.3 °C
Lighting:	16/8 light/dark phases (on average 141 µE $*m^{-2}*s^{-1}$)
Treatments:	control (untreated test medium) test item
Test concentration (nominal)*:	7.44, 23.8, 76.3, 244.0, 781.3, 2500.0 µg/L test item equivalent to 3.29, 10.6, 33.8, 108.0, 345.8, 1106.7 µg/L Aclonifen 0.37, 1.19, 3.82, 12.2, 39.2, 125.3 µg/L Flufenacet
Test concentration (mean measured):	2.53, 9.42, 29.98, 102.3, 316.7, 1082.8 µg/L Aclonifen 5.88, 21.5, 68.6, 232.4, 722.0, 2451.5 µg/L test item, recalculated
Exposure time:	14 days (semi-static test procedure)
Biological observations:	*day 0 and 14:* main shoot length, length and number of lateral branches *day 7 and 14:* changes in plant development *day 0 and 14:*

	fresh and dry weight *day 0, 14:* observation root development
Statistics:	NOEC/LOEC: Williams t-test; Welch's t-test (alpha = 0.05, one-sided smaller) ECx: Probit analysis using linear max. likelihood and linear weighted regression Statistical program: ToxRat Professional Version 3.3 (20.10.2018)

The study was performed in compliance with the principles of GLP.

* nominal test concentrations based on the weighed amount of test item (mean values of day 0 and 7)

Dates of work:	biological phase (exposure time):	
	experimental start date:	11.05.2021
	experimental completion date (biological, determination dry weight):	02.06.2021
	analytical phase:	
	experimental start:	21.06.2021
	experimental completion date:	16.07.2021

Findings:

The test was valid based on doubling of total shoot length and fresh weight in control plants: required factor: 2, achieved: factor 5.0 for total shoot length and factor 7.0 for fresh weight. Control plants did not show any visual symptoms of chlorosis and were visibly free from contamination by other organisms such as algae and/or bacterial films on the plants. No observations of the latter were made at the surface of the sediment and in test medium. The mean coefficient of variation for yield, based on measurements of shoot fresh weight in control cultures, does not exceed 35% (achieved: 7.2%).

The measured concentrations of Aclonifen in the overlying water were within ranges of 81.4 – 115.1% of nominal concentrations in the freshly prepared test solutions at the start of the test and at the test solution renewal and within a range of 67.3 – 90.2% in the spent solutions at each renewal of the test solutions and at the test end (day 14) based on nominal values.

The measured concentrations of Flufenacet in the overlying water were within ranges of 89.0 – 107.2% of nominal concentrations in the freshly prepared test solutions at the start of the test and at each renewal and within a range of 87.7 – 97.0% in the spent solutions at each renewal of the test solutions and at the test end (day 14) based on nominal values.

Therefore, the calculated study endpoints are based on nominal and on the mean measured concentrations for the active substance Aclonifen as well as on the test item, recalculated, because the recoveries for Aclonifen were not between 80 and 120% of nominal.

Table 7: Effects of GLOB1310aH on yield and growth of *Myriophyllum spicatum* for biomass (fresh and dry weight)

Treatment Group µg/L test item, nominal	**Biomass (fresh weight)** replicate mean day 14 (mg)	**Biomass (dry weight)** replicate mean day 14 (mg)	**% Inhibition**			
			Average specific growth rate (% I_r)		**Yield (% I_y)**	
			Biomass (fresh weight)	Biomass (dry weight)	Biomass (fresh weight)	Biomass (dry weight)
Control	667.3	49.4	n.r.	n.r.	n.r.	n.r.
7.44	677.0	50.9	-0.8[1]	-3.0[1]	-1.7[1]	-4.3[1]

23.8	569.4	42.7	8.4 +	12.0	17.1 +	19.0 +
76.3	421.5	33.8	24.1 +	31.0 +	43.0 +	44.2 +
244.0	335.6	29.2	35.6 +	41.8 +	58.0 +	57.3 +
781.3	279.6	33.2	44.9 +	32.0 +	67.8 +	46.0 +
2500.0	194.5	28.1	63.6 +	45.6 +	82.7 +	60.6 +

n.r. – not relevant
+ significantly different to the control
(Williams t-test, alpha = 0.05, one-sided smaller)
[1] negative values indicate higher growth compared to the control

Table 8: Effects of GLOB1310aH on growth rate and yield of *Myriophyllum spicatum* for main shoot length

Treatment group µg/L test item, (nominal)	**Main shoot length** replicate mean day 14 (cm)	**% Inhibition**	
		Average specific growth rate **(% I_r)**	**Yield** **(% I_y)**
Control	27.5	n.r.	n.r.
7.44	28.4	5.1	-0.5[1]
23.8	26.2	7.1 +	8.3 +
76.3	21.2	20.4 +	31.8 +
244.0	19.4	29.4 +	42.0 +
781.3	12.2	70.7 +	81.0 +
2500.0	9.9	87.2 +	92.6 +

+ Significantly different to the control (Welch's-t-test; alpha = 0.05, one-sided smaller)
n.r. – not relevant
[1] negative values indicate higher growth compared to the control

Table 9: Effects of GLOB1310aH on growth rate and yield of *Myriophyllum spicatum* for total shoot length

Treatment group µg/L test item, (nominal)	**Total shoot length** replicate mean day 14 (cm)	**% Inhibition**	
		Average specific growth rate **(% I_r)**	**Yield** **(% I_y)**
Control	39.7	n.r.	n.r.
7.44	40.6	4.3	-0.4[1]
23.8	34.0	12.2 +	18.9 +
76.3	25.6	26.9 +	44.1 +
244.0	21.4	39.5 +	58.2 +
781.3	14.1	68.0 +	82.1 +
2500.0	10.7	85.1 +	92.8 +

+ significantly different to the control (Williams t-test; alpha = 0.05, one-sided smaller)
[1] negative values indicate higher growth compared to control
n.r. – not relevant

Table 10: EC_x-values, LOEC and NOEC values of GLOB1310aH for growth rate and yield based on biomass (fresh and dry weight) of *Myriophyllum spicatum* at test end after 14 days

Effect concentration	**GLOB1310aH, µg/L**			
	Average specific growth rate		Yield	
	biomass (fresh weight)	biomass (dry weight)	biomass (fresh weight)	biomass (dry weight)
NOEC				
test item, nominal	**7.44**	**23.8**	**7.44**	**7.44**
Aclonifen, nominal	**3.29**	**10.6**	**3.29**	**3.29**
Flufenacet, nominal	**0.37**	**1.19**	**0.37**	**0.37**

Aclonifen, mean measured	**2.53**	**9.42**	**2.53**	**2.53**
test item, mean measured recalculated	**5.88**	**21.5**	**5.88**	**5.88**
LOEC				
test item, nominal	**23.8**	**76.3**	**23.8**	**23.8**
Aclonifen, nominal	**10.6**	**33.8**	**10.6**	**10.6**
Flufenacet, nominal	**1.19**	**3.82**	**1.19**	**1.19**
Aclonifen, mean measured	**9.42**	**30.0**	**9.42**	**9.42**
test item, mean measured recalculated	**21.5**	**68.6**	**21.5**	**21.5**

Table 4: EC_X-values, LOEC and NOEC values of GLOB1310aH for growth rate and yield based on biomass (fresh and dry weight) of *Myriophyllum spicatum* at test end after 14 days (continued)

Effect concentration	**GLOB1310aH, µg/L**			
	Average specific growth rate		Yield	
	biomass (fresh weight)	biomass (dry weight)	biomass (fresh weight)	biomass (dry weight)
EC_{10} 95 % confidence limits (lower – upper)	**E_rC_{10}**	**E_rC_{10}**	**E_yC_{10}**	**E_yC_{10}**
test item, nominal	**20.7** (11.0 – 33.3)	**8.84** (1.69 – 22.2)	**8.11** (3.63 – 14.3)	**5.75** (1.02 – 15.0)
Aclonifen, nominal	**9.18** (4.88 – 14.7)	**3.91** (0.75 – 9.82)	**3.59** (1.61 – 6.32)	**2.55** (0.45 – 6.65)
Flufenacet, nominal	**1.04** (0.55 – 1.67)	**0.44** (0.08 – 1.11)	**0.41** (0.18 – 0.72)	**0.29** (0.05 – 0.75)
Aclonifen, mean measured	**7.96** (4.20 – 12.9)	**3.24** (0.61 – 8.27)	**3.02** (1.33 – 5.37)	**2.08** (0.37 – 5.52)
test item, mean measured recalculated	**18.3** (9.64 – 29.5)	**7.47** (1.41 – 19.0)	**6.94** (3.07 – 12.4)	**4.80** (0.85 – 12.7)
EC_{20} 95 % confidence limits (lower – upper)	**E_rC_{20}**	**E_rC_{20}**	**E_yC_{20}**	**E_yC_{20}**

test item, nominal	**75.8** (49.9 – 105.2)	**87.8** (39.3 – 180.0)	**23.8** (13.4 – 36.3)	**30.4** (10.7 – 61.6)
Aclonifen, nominal	**33.6** (22.1 – 46.6)	**38.9** (17.4 – 79.7)	**10.5** (5.91 – 16.1)	**13.4** (4.72 – 27.3)
Flufenacet, nominal	**3.80** (2.50 – 5.27)	**4.40** (1.97 – 9.02)	**1.19** (0.67 – 1.82)	**1.52** (0.53 – 3.09)
Aclonifen, mean measured	**30.0** (19.6 – 41.7)	**34.3** (15.2 – 71.3)	**9.10** (5.05 – 14.0)	**11.5** (4.00 – 23.7)
test item, mean measured recalculated	**68.5** (44.9 – 95.4)	**78.6** (34.7 – 163.1)	**20.9** (11.6 – 32.1)	**26.5** (9.21 – 54.3)

Table 4: EC_X-values, LOEC and NOEC values of GLOB1310aH for growth rate and yield based on biomass (fresh and dry weight) of *Myriophyllum spicatum* at test end after 14 days (continued)

Effect concentration	**GLOB1310aH, µg/L**			
	Average specific growth rate		Yield	
	biomass (fresh weight)	biomass (dry weight)	biomass (fresh weight)	biomass (dry weight)
EC_{50} 95 % confidence limits (lower – upper)	**E_rC_{50}**	**E_rC_{50}**	**E_yC_{50}**	**E_yC_{50}**
test item, nominal	**904.3** (701.4 – 1213.9)	**> 2500.0**	**186.4** (138.3 – 252.5)	**733.9** (340.3 – 2542.0)
Aclonifen, nominal	**400.3** (310.5 – 537.4)	**> 1106.7**	**82.5** (61.2 – 111.8)	**324.9** (150.6 – 1125.3)
Flufenacet, nominal	**45.3** (35.2 – 60.8)	**> 125.3**	**9.34** (6.93 – 12.7)	**36.8** (17.1 – 127.4)
Aclonifen, mean measured	**378.4** (293.0 – 509.0)	**> 1082.8**	**75.3** (55.6 – 102.2)	**304.6** (139.3 – 1065.7)
test item, mean measured recalculated	**859.9** (665.8 – 1156.3)	**> 2451.5**	**171.7** (127.0 – 233.1)	**692.7** (317.2 – 2420.7)

Table 11: EC_X-values, LOEC and NOEC values of GLOB1310aH for growth rate and yield based on main and total shoot length of *Myriophyllum spicatum* at test end after 14 days

Effect concentration	GLOB1310aH, µg/L			
	Average specific growth rate		Yield	
	main shoot length	total shoot length	main shoot length	total shoot length
NOEC				
test item, nominal	**7.44**	**7.44**	**7.44**	**7.44**
Aclonifen, nominal	**3.29**	**3.29**	**3.29**	**3.29**
Flufenacet, nominal	**0.37**	**0.37**	**0.37**	**0.37**
Aclonifen, mean measured	**2.53**	**2.53**	**2.53**	**2.53**
test item, mean measured recalculated	**5.88**	**5.88**	**5.88**	**5.88**

Table 5: EC_X-values, LOEC and NOEC values of GLOB1310aH for growth rate and yield based on main and total shoot length of *Myriophyllum spicatum* at test end after 14 days (continued)

Effect concentration	GLOB1310aH, µg/L			
	Average specific growth rate		Yield	
	main shoot length	total shoot length	main shoot length	total shoot length
LOEC				
test item, nominal	**23.8**	**23.8**	**23.8**	**23.8**
Aclonifen, nominal	**10.6**	**10.6**	**10.6**	**10.6**
Flufenacet, nominal	**1.19**	**1.19**	**1.19**	**1.19**
Aclonifen, mean measured	**9.42**	**9.42**	**9.42**	**9.42**
test item, mean measured recalculated	**21.5**	**21.5**	**21.5**	**21.5**
EC_{10} 95 % confidence limits (lower – upper)	**E_rC_{10}**	**E_rC_{10}**	**E_yC_{10}**	**E_yC_{10}**
test item, nominal	**48.6** (29.9 – 69.3)	**21.9** (15.2 – 29.4)	**25.6** (15.7 – 36.8)	**10.7** (6.75 – 15.4)
Aclonifen, nominal	**21.5**	**9.68**	**11.3**	**4.75**

	(13.3 – 30.7)	(6.73 – 13.0)	(6.93 – 16.3)	(2.99 – 6.81)
Flufenacet, nominal	**2.43** (1.50 – 3.47)	**1.10** (0.76 – 1.48)	**1.28** (0.78 – 1.85)	**0.54** (0.34 – 0.77)
Aclonifen, mean measured	**19.3** (11.5 – 28.1)	**8.45** (5.74 – 11.6)	**9.94** (5.86 – 14.6)	**4.02** (2.48 – 5.84)
test item, mean measured recalculated	**44.1** (26.3 – 64.0)	**19.4** (13.2 – 26.5)	**22.8** (13.5 – 33.5)	**9.24** (5.71 – 13.4)

Table 5: EC_X-values, LOEC and NOEC values of GLOB1310aH for growth rate and yield based on main and total shoot length of *Myriophyllum spicatum* at test end after 14 days (continued)

Effect concentration	**GLOB1310aH, µg/L**			
	Average specific growth rate		Yield	
	main shoot length	total shoot length	main shoot length	total shoot length
EC_{20} 95 % confidence limits (lower – upper)	**E_rC_{20}**	**E_rC_{20}**	**E_yC_{20}**	**E_yC_{20}**
test item, nominal	**100.1** (70.4 – 130.9)	**55.1** (42.4 – 68.7)	**54.6** (38.1 – 72.0)	**25.5** (18.2 – 33.5)
Aclonifen, nominal	**44.3** (31.2 – 58.0)	**24.4** (18.8 – 30.4)	**24.2** (16.9 – 31.9)	**11.3** (8.03 – 14.8)
Flufenacet, nominal	**5.02** (3.53 – 6.56)	**2.76** (2.12 – 3.44)	**2.74** (1.91 – 3.61)	**1.28** (0.91 – 1.68)
Aclonifen, mean measured	**40.3** (27.6 – 53.5)	**21.7** (16.4 – 27.5)	**21.6** (14.7 – 29.0)	**9.78** (6.86 – 13.0)
test item, mean measured recalculated	**91.9** (63.2 – 121.8)	**49.7** (37.7 – 62.7)	**49.3** (33.6 – 66.1)	**22.4** (16.8 – 29.8)
EC_{50} 95 % confidence limits (lower – upper)	**E_rC_{50}**	**E_rC_{50}**	**E_yC_{50}**	**E_yC_{50}**
test item, nominal	**399.1** (328.5 – 486.4)	**323.2** (277.8 – 377.2)	**233.0** (190.5 – 285.2)	**133.7** (110.4 – 161.9)
Aclonifen, nominal	**176.7** (145.4 – 215.3)	**143.1** (123.0 – 167.0)	**103.1** (84.3 – 126.3)	**59.2** (48.9 – 71.6)
Flufenacet, nominal	**20.0**	**16.2**	**11.7**	**6.70**

	(16.5 – 24.4)	(13.9 – 18.9)	(9.55 – 14.3)	(5.53 – 8.11)
Aclonifen, mean measured	**164.6** (134.0 – 202.8)	**132.5** (113.0 – 156.0)	**95.1** (76.8 – 117.8)	**53.7** (44.1 – 65.3)
test item, mean measured recalculated	**374.6** (305.3 – 461.0)	**301.9** (257.5 – 354.9)	**216.6** (175.2 – 268.0)	**122.5** (100.7 – 149.1)

Conclusion:

A growth inhibition test was performed to assess the effects of the test item GLOB1310aH to the rooted aquatic macrophyte *Myriophyllum spicatum* during 14 days of exposure.

The calculated study endpoints are based on nominal concentrations for test item and the active substances Aclonifen and Flufenacet as well as on the mean measured concentrations for Aclonifen and for test item (recalculated).

Based on recalculated test item concentrations (mean measured) the EC_{50} value based on yield was 122.5 µg/L test item, recalculated for total shoot length. The EC_{50} value based on growth rate was 301.9 µg/L test item calculated for total shoot length.

Based on recalculated test item concentrations (mean measured) the EC_{50} value for shoot length based on yield was 216.6 µg/L test item, recalculated. The EC_{50} value based on growth rate was 374.6 µg/L test item, recalculated.

Based on recalculated test item concentrations (mean measured) the EC_{50} value for fresh weight based on yield was 171.7 µg/L test item, recalculated. The EC_{50} value based on growth rate was 859.9 µg/L test item, recalculated.

Based on recalculated test item concentrations (mean measured) the EC_{50} value for dry weight based on yield was 692.7 µg/L test item, recalculated. The EC_{50} value based on growth rate was higher than 2451.5 µg/L test item, recalculated.

Comments of zRMS:	The study was performed according to OECD TG 201 and principles of GLP. The validity criteria are met. The biomass in the control cultures increased exponetially by a factor of 70.6 within the 72 hours test period (factor 16 after 72 hours is required according to guideline OECD Guideline 201 (2011)). The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 20.6% (not exceeding 35%). The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was 1.4% and did not exceed 7%. The study is considered acceptable and suitable for the risk assessment. The results for test item refer to nominal concentrations and the results for active substance refer to the mean measured concentrations since the measured concentrations were not within 80 to 120% of nominal.

Reference:	KCP 10.2.1
Report	Effects of GLOB1310aH on *Desmodesmus subspicatus* in an algal growth inhibition test. Juckeland, D., 2021d. Study No. 21 48 AAL 0017
Guideline(s):	Yes (OECD 201 (2011))

Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

SUMMARY

Study title:	Effects of GLOB1310aH on *Desmodesmus subspicatus* in an algal growth inhibition test
Guidelines:	OECD 201 (2011)
BioChem project No.:	21 48 AAL 0017
Test item:	GLOB1310aH Batch no.: GLO-20F-2306A Content of active substances (analysed) Aclonifen: 543.7 g/L Flufenacet: 61.55 g/L
Test species:	Freshwater green alga – *Desmodesmus subspicatus* HILSE
Test system:	Exposure of *Desmodesmus subspicatus* to the test item applied once in test medium (static conditions)
Test conditions:	Temperature: 22.9 – 24.0°C Continuous light: (on average 75 µE m^{-2} s^{-1})
Treatments:	Control (untreated test medium) GLOB1310aH
Test concentration (nominal):	23.8, 42.9, 77.2, 138.9, 250.0 µg/L test item equivalent to 10.5, 19.0, 34.2, 61.5, 110.7 µg/L Aclonifen 1.19, 2.15, 3.87, 6.96, 12.5 µg/L Flufenacet
Test concentration (geometrical mean measured):	6.35, 13.56, 26.72, 51.27, 96.69 µg/L Aclonifen 1.02, 2.06, 3.79, 7.12, 13.31 µg/L Flufenacet 15.0, 31.7, 61.9, 118.5, 223.2 µg/L test item, recalculated
Exposure time:	72 hours (static test procedure)
Biological observations:	Number of cells: after 24, 48 and 72 hours
Statistics:	EC_x-values: non-linear regression: 3-param. normal CDF*, linear regression (Probit) for mean measured concentrations LOEC/NOEC: Welch-t-test; Williams t-test, alpha = 0.05, one-sided smaller Statistical program: ToxRat Professional Version 3.3.0 (20.10.2018)
Dates of work:	Biological phase: experimental start date: 18.05.2021 experimental completion date: 21.05.2021

Analytical phase:	
experimental start:	01.07.2021
experimental completion:	09.07.2021

* CDF - cumulative distribution function

Findings

Measured concentrations of Aclonifen in test solutions were within a range of 64.6 to 82.0% of nominal values at the test start and after 72 hours the concentrations ranged from 56.2 to 94.9% of nominal in spent test solutions.

Measured concentrations of Flufenacet in test solutions were within a range of 94.8 to 106.6% of nominal values at the test start and after 72 hours the concentrations ranged from 76.7 to 106.0% of nominal in spent test solutions.

Therefore, the calculated endpoints are based on the nominal concentrations for the test item and mean measured concentration for Aclonifen and Flufenacet, since the measured concentrations for this active substance were not within 80 to 120% of nominal at the test end. Furthermore the endpoints based on the recalculated test item concentrations.

Table 12: Effects on growth rate and yield of *Desmodesmus subspicatus*

Effect concentration	**GLOB1310aH, µg/L**	
	Average specific growth rate inhibition	**Yield inhibition**
	0 – 72 h after application	
NOEC		
Test item, nominal	**23.8**	**23.8**
Aclonifen, nominal	**10.54**	**10.54**
Flufenacet, nominal	**1.19**	**1.19**
Aclonifen, mean measured	**6.35**	**6.35**
Flufenacet, mean measured	**1.02**	**1.02**
test item, mean measured recalculated	**15.0**	**15.0**
LOEC		
Test item, nominal	**42.9**	**42.9**
Aclonifen, nominal	**18.98**	**18.98**
Flufenacet, nominal	**2.15**	**2.15**
Aclonifen, mean measured	**13.56**	**13.56**
Flufenacet, mean measured	**2.06**	**2.06**
test item, mean measured recalculated	**31.7**	**31.7**

Table 1: Effects on growth rate and yield of *Desmodesmus subspicatus* (continued)

Effect concentration	GLOB1310aH, µg/L nominal	
	Average specific growth rate inhibition	**Yield inhibition**
	0 – 72 h after application	
EC_{10} and 95% confidence intervals (lower – upper)	**E_rC_{10}**	**E_yC_{10}**
test item, nominal	**36.3** (30.3 - 43.4)	**22.3** (16.8 – 29.6)
Aclonifen, nominal	**16.1** (13.4 - 19.2)	**9.87** (7.44 - 13.1)
Flufenacet, nominal	**1.82** (1.52 - 2.17)	**1.12** (0.84 - 1.48)
Aclonifen, mean measured	**11.36** (9.33 – 13.83)	**6.09** (4.46 – 8.33)
Flufenacet, mean measured	**1.69** (1.41 – 2.03)	**0.99** (0.74 - 1.32)
test item, mean measured recalculated	**26.5** (21.8 – 32.2)	**14.4** (10.6 – 19.6)
EC_{20} and 95% confidence intervals (lower – upper)	**E_rC_{20}**	**E_yC_{20}**
test item, nominal	**55.3** (46.7 - 65.6)	**29.5** (22.6 - 38.5)
Aclonifen, nominal	**24.5** (20.7 - 29.0)	**13.1** (10.0 - 17.0)
Flufenacet, nominal	**2.77** (2.34 - 3.29)	**1.48** (1.13 - 1.93)
Aclonifen, mean measured	**18.19** (15.11 – 21.94)	**8.49** (6.32 – 11.4)
Flufenacet, mean measured	**2.66** (2.24 – 3.16)	**1.34** (1.02 – 1.75)
test item, mean measured recalculated	**42.3** (35.2 – 50.9)	**20.0** (14.9 – 26.7)

Table 1: Effects on growth rate and yield of *Desmodesmus subspicatus* (continued)

Effect concentration	**GLOB1310aH, µg/L nominal**	
	Average specific growth rate inhibition	**Yield inhibition**
	0 – 72 h after application	
EC_{50} and 95% confidence intervals (lower – upper)	**E_rC_{50}**	**E_yC_{50}**
Test item, nominal	**123.7** (100.2 - 151.9)	**50.3** (36.5 - 69.3)
Aclonifen, nominal	**54.8** (44.4 - 67.2)	**22.3** (16.2 - 30.7)
Flufenacet, nominal	**6.20** (5.02 - 7.61)	**2.52** (1.83 - 3.47)
Aclonifen, mean measured	**44.82** (35.56 – 56.15)	**16.0** (11.3 – 22.8)
Flufenacet, mean measured	**6.28** (5.07 – 7.74)	**2.39** (1.73 – 3.31)
test item, mean measured recalculated	**103.7** (82.5 – 129.6)	**37.4** (26.3 – 53.0)

Calculations were done using unrounded values

Table 13: Observations

Treatment group	**% Inhibition**	
µg/L test item, nominal	Average specific growth rate	Yield
	0 - 72 h after application	
Control	n.r.	n.r.
9.82	0.0	0.0
21.6	16.2 +	49.2 +
47.5	29.0 +	70.8 +
138.9	55.1 +	91.0 +
250.0	76.5 +	97.2 +

+ significantly different from control (Welch-t-test for growth rate, Williams t-test for yield)
alpha = 0.05, one-sided smaller), n.r. – not relevant

Validity criteria

The biomass in the control cultures increased exponentially by a factor of 60.3 within the 72 hours test period (factor 16 after 72 hours is required according to guideline OECD Guideline 201 (2011)). The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 17.3% (not exceeding 35%). The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was 1.0% and did not exceed 7%.

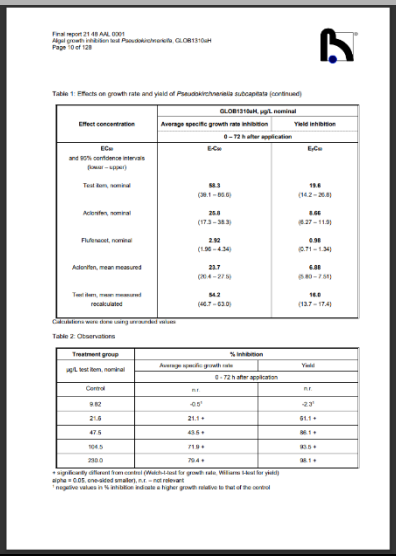
Conclusion

A growth inhibition test was performed to assess the effects of the test item GLOB1310aH to a freshwater green alga (*Desmodesmus subspicatus*) during 72 hours of exposure.

The analysis of the test solutions demonstrates that the organisms were exposed to the appropriate concentration of test material at study initiation. The calculated endpoints are based on the nominal test concentrations for the test item and based on the geometrical mean measured concentrations for Aclonifen and Flufenacet since the measured concentrations for this active substance were not within 80 to 120% of nominal.

In a 72-hour static test in which *Desmodesmus subspicatus* were exposed to GLOB1310aH, based on nominal concentrations the 72 h E_rC_{50} (growth rate) was 123.7 µg/L test item (equivalent to 44.48 µg/L Aclonifen and 6.30 µg/L Flufenacet mean measured) and the 72 h E_yC_{50} (yield) was 50.3 µg/L test item (equivalent to 15.88 µg/L Aclonifen and 2.40 µg/L Flufenacet mean measured).

Comments of zRMS:	The study was performed according to OECD TG 201 and principles of GLP. The validity criteria are met. The biomass in the control cultures increased exponentially by a factor of at least 16 within the 72-hour test period (being 80.2). The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures did not exceed 35% (being 8.9%). The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures did not exceed 7% (being 0.9%). The study is considered acceptable and suitable for the risk assessment. According to the Final report 21 48 AAL 0017: - based on the mean measured concentration the 72h ErC50 (growth rate) was 54.2 µg/L test item, recalculated, - based on the nominal concentration the 72h ErC50 (growth rate) was 58.3 µg/L test item.



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Algal growth inhibition test *Pseudokirchneriella*, GLOB1310aH
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Table 1: Effects on growth rate and yield of *Pseudokirchneriella subcapitata* (continued)

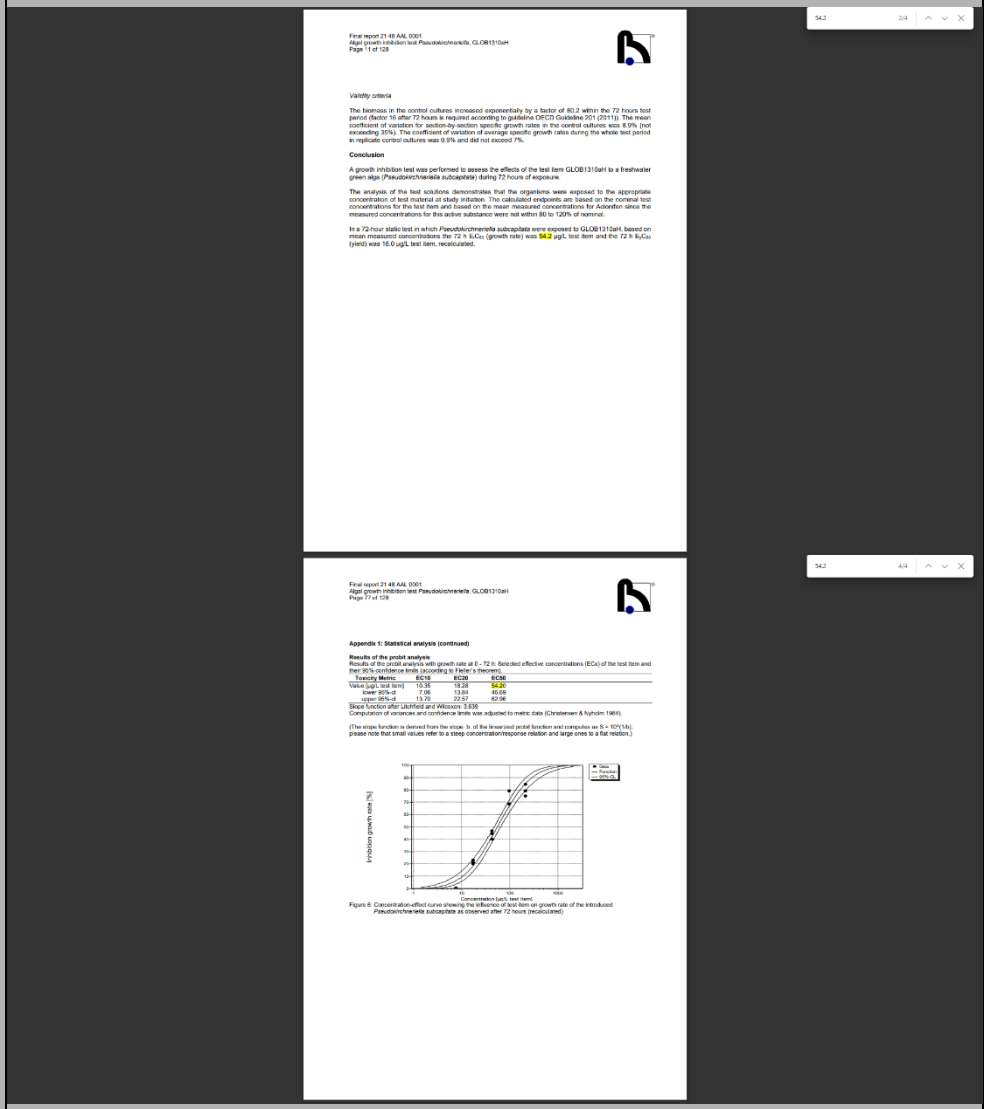
Effect concentration	GLOB1310aH, µg/L nominal	
	Average specific growth rate inhibition	Yield inhibition
	0 – 72 h after application	
ECx and 95% confidence intervals (lower – upper)	E_rCx	E_yCx
Test item, nominal	**58.3** (36.1 – 85.0)	**19.6** (14.2 – 26.8)
Aclonifen, nominal	**25.0** (17.3 – 38.3)	**8.66** (6.27 – 11.9)
Flufenacet, nominal	**2.92** (1.96 – 4.34)	**0.98** (0.71 – 1.34)
Aclonifen, mean measured	**23.7** (20.4 – 27.5)	**6.88** (5.80 – 7.91)
Test item, mean measured recalculated	**54.2** (46.7 – 63.0)	**16.0** (13.7 – 17.4)

Calculations were done using unrounded values

Table 2: Observations

Treatment group	% inhibition	
µg/L test item, nominal	Average specific growth rate	Yield
	0 – 72 h after application	
Control	n.r.	n.r.
9.82	-0.5[1]	-2.3[1]
21.6	21.1 *	51.1 *
47.5	43.6 *	86.1 *
104.5	71.9 *	93.0 *
230.0	79.4 *	96.1 *

* significantly different from control (Welch t-test for growth rate, Williams t-test for yield)
alpha = 0.05, one-sided smaller), n.r. = not relevant
[1] negative values in % inhibition indicate a higher growth relative to that of the control



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Algal growth inhibition test Pseudokirchneriella, GLOB1310aH
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Validity criteria

The biomass in the control cultures increased exponentially by a factor of 80.2 within the 72 hours test period (factor 16 after 72 hours is required according to guideline OECD Guideline 201 (2011)). The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 8.9% (not exceeding 35%). The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was 0.9% and did not exceed 7%.

Conclusion

A growth inhibition test was performed to assess the effects of the test item GLOB1310aH to a freshwater green alga (*Pseudokirchneriella subcapitata*) during 72 hours of exposure.

The analysis of the test solutions demonstrates that the organisms were exposed to the appropriate concentration of test material at study initiation. The calculated endpoints are based on the nominal test concentrations for the test item and based on the mean measured concentrations for Aclonifen since the measured concentrations for this active substance were not within 80 to 120% of nominal.

In a 72-hour static test in which *Pseudokirchneriella subcapitata* were exposed to GLOB1310aH, based on mean measured concentrations the 72 h E_bC_{50} (growth rate) was **54.2** µg/L test item and the 72 h E_yC_{50} (yield) was 16.0 µg/L test item, recalculated.

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Appendix 1: Statistical analysis (continued)

Results of the probit analysis

Results of the probit analysis with growth rate at 0 - 72 h: Selected effective concentrations (EC_x) of the test item and their 95%-confidence limits (according to Fieller's theorem).

Toxicity Metric	EC10	EC20	EC50
Value [µg/L test item]	10.35	18.28	**54.20**
lower 95%-cl	7.06	13.84	46.68
upper 95%-cl	13.70	22.57	62.98

Slope function after Litchfield and Wilcoxon: 3.636
Computation of variances and confidence limits was adjusted to metric data (Christensen & Nyholm 1984).

(The slope function is derived from the slope, b, of the linearized probit function and computes as S = 10^(1/b); please note that small values refer to a steep concentration/response relation and large ones to a flat relation.)

Figure 6: Concentration-effect curve showing the influence of test item on growth rate of the introduced *Pseudokirchneriella subcapitata* as observed after 72 hours (recalculated)

The worst-case endpoint at 54.2 µg/L test item was used for the risk assessment by Applicant.

Reference:	KCP 10.2.1
Report	Effects of GLOB1310aH on *Pseudokirchneriella subcapitata* in an algal growth inhibition test. Juckeland, D., 2021e. Study No. 21 48 AAL 0017
Guideline(s):	Yes (OECD 201 (2011))
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

- **SUMMARY**

Study title:	Effects of GLOB1310aH on *Pseudokirchneriella subcapitata* in an algal growth inhibition test

Guidelines:	OECD 201 (2011)
BioChem project No.:	21 48 AAL 0001
Test item:	GLOB1310aH Batch no.: GLO-20F-2306A Content of active substances (analysed) Aclonifen: 543.7 g/L Flufenacet: 61.55 g/L
Test species:	Freshwater green alga – *Pseudokirchneriella subcapitata* KORSHIKOV
Test system:	Exposure of *Pseudokirchneriella subcapitata* to the test item applied once in test medium (static conditions)
Test conditions:	Temperature: 22.7 – 24.0°C Continuous light: (on average 75 µE m^{-2} s^{-1})
Treatments:	Control (untreated test medium) GLOB1310aH
Test concentration (nominal):	9.82, 21.6, 47.5, 104.5, 230.0 µg/L test item equivalent to 4.35, 9.56, 21.0, 46.3, 101.8 µg/L Aclonifen 0.49, 1.08, 2.38, 5.24, 11.5 µg/L Flufenacet
Test concentration (mean measured):	3.24, 7.60, 18.9, 42.4, 93.5 µg/L Aclonifen
Exposure time:	72 hours (static test procedure)
Biological observations:	Number of cells: after 24, 48 and 72 hours
Statistics:	EC_x-values: non-linear regression: 3-param. normal CDF*, linear regression (Probit) for mean measured concentrations LOEC/NOEC: Welch-t-test; Williams t-test, alpha = 0.05, one-sided smaller Statistical program: ToxRat Professional Version 3.3 (20.10.2018)
Dates of work:	Biological phase: experimental start date: 04.05.2021 experimental completion date: 07.05.2021 Analytical phase: experimental start: 04.06.2021 experimental completion: 04.06.2021

* CDF - cumulative distribution function

Findings

Measured concentrations of Aclonifen in test solutions were within a range of 80.1 to 104.7% of nominal values at the test start and after 72 hours the concentrations ranged from 62.7 to 83.4% of nominal in spent test solutions.

Measured concentrations of Flufenacet in test solutions were within a range of 96.7 to 99.8% of nominal values at the test start and after 72 hours the concentrations ranged from 87.6 to 106.4% of nominal in spent test solutions.

Therefore, the calculated endpoints are based on the nominal concentrations for the test item and mean

measured concentration for Aclonifen, since the measured concentrations for this active substance were not within 80 to 120% of nominal at the test end.

Table 14: Effects on growth rate and yield of *Pseudokirchneriella subcapitata*

Effect concentration	GLOB1310aH, µg/L	
	Average specific growth rate inhibition	Yield inhibition
	0 – 72 h after application	
NOEC		
Test item, nominal	**9.82**	**9.82**
Aclonifen, nominal	**4.35**	**4.35**
Flufenacet, nominal	**0.49**	**0.49**
Aclonifen, mean measured	**3.24**	**3.24**
LOEC		
Test item, nominal	**21.6**	**21.6**
Aclonifen, nominal	**9.56**	**9.56**
Flufenacet, nominal	**1.08**	**1.08**
Aclonifen, mean measured	**7.60**	**7.60**

Table 1: Effects on growth rate and yield of *Pseudokirchneriella subcapitata* (continued)

Effect concentration	GLOB1310aH, µg/L nominal	
	Average specific growth rate inhibition	Yield inhibition
	0 – 72 h after application	
EC_{10} and 95% confidence intervals (lower – upper)	**E_rC_{10}**	**E_yC_{10}**
test item, nominal	**11.9** (8.39 – 16.9)	**11.5** (8.72 – 15.1)
Aclonifen, nominal	**5.27** (3.71 – 7.47)	**5.08** (3.86 – 6.69)
Flufenacet, nominal	**0.60** (0.42 – 0.85)	**0.58** (0.44 – 0.76)
Aclonifen, mean measured	**4.45** (3.04 – 5.89)	**3.98** (2.10 – 4.96)

EC_{20} and 95% confidence intervals (lower – upper)	**E_rC_{20}**	**E_yC_{20}**
test item, nominal	**20.5** (14.8 – 28.5)	**13.8** (10.6 – 17.9)
Aclonifen, nominal	**9.09** (6.54 – 12.6)	**6.10** (4.71 – 7.93)
Flufenacet, nominal	**1.03** (0.74 – 1.43)	**0.69** (0.53 – 0.90)
Aclonifen, mean measured	**7.89** (5.97 – 9.74)	**4.80** (3.05 – 5.65)
EC_{50} and 95% confidence intervals (lower – upper)	**E_rC_{50}**	**E_yC_{50}**
Test item, nominal	**58.3** (39.1 – 86.6)	**19.6** (14.2 – 26.8)
Aclonifen, nominal	**25.8** (17.3 – 38.3)	**8.66** (6.27 – 11.9)
Flufenacet, nominal	**2.92** (1.96 – 4.34)	**0.98** (0.71 – 1.34)
Aclonifen, mean measured	**23.6** (20.3 – 27.3)	**6.87** (5.97 – 7.54)

Calculations were done using unrounded values

Table 15: Observations

Treatment group	**% Inhibition**	
µg/L test item, nominal	Average specific growth rate	Yield
	0 - 72 h after application	
Control	n.r.	n.r.
9.82	-0.5[1]	-2.3[1]
21.6	21.1 +	61.1 +
47.5	43.5 +	86.1 +
104.5	71.9 +	93.5 +
230.0	79.4 +	98.1 +

+ significantly different from control (Welch-t-test for growth rate, Williams t-test for yield)
alpha = 0.05, one-sided smaller), n.r. – not relevant
[1] negative values in % inhibition indicate a higher growth relative to that of the control

Validity criteria

The biomass in the control cultures increased exponentially by a factor of 80.2 within the 72 hours test period (factor 16 after 72 hours is required according to guideline OECD Guideline 201 (2011)). The mean coefficient of variation for section-by-section specific growth rates in the control cultures was 8.9% (not exceeding 35%). The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures was 0.9% and did not exceed 7%.

Conclusion

A growth inhibition test was performed to assess the effects of the test item GLOB1310aH to a freshwater green alga (*Pseudokirchneriella subcapitata*) during 72 hours of exposure.

The analysis of the test solutions demonstrates that the organisms were exposed to the appropriate concentration of test material at study initiation. The calculated endpoints are based on the nominal test concentrations for the test item and based on the mean measured concentrations for Aclonifen since the measured concentrations for this active substance were not within 80 to 120% of nominal.

In a 72-hour static test in which *Pseudokirchneriella subcapitata* were exposed to GLOB1310aH, based on nominal concentrations the 72 h E_rC_{50} (growth rate) was 58.3 µg/L test item (equivalent to 23.6 µg/L Aclonifen, mean measured and 2.92 µg/L Flufenacet) and the 72 h E_yC_{50} (yield) was 19.6 µg/L test item (equivalent to 6.87 µg/L Aclonifen, mean measured and 0.98 µg/L Flufenacet).

A 2.2.2 KCP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

A 2.2.3 KCP 10.2.3 Further testing on aquatic organisms

A 2.3 KCP 10.3 Effects on arthropods

A 2.3.1 KCP 10.3.1 Effects on bees

A 2.3.1.1 KCP 10.3.1.1 Acute toxicity to bees

A 2.3.1.1.1 KCP 10.3.1.1.1 Acute oral toxicity to bees

Comments of zRMS:	The study was conducted to OECD guidelines 213 and 214 and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.1.1.1 and KCP 10.3.1.1.2
Report	Acute toxicity of GLOB1310aH to the honeybee *Apis mellifera* L. under laboratory conditions. Franke, M., 2021.Study No. 21 48 BAA 0001
Guideline(s):	Yes (OECD 213 (1998, OECD 214 (1998)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication	Not a vertebrate study

(if vertebrate study)

Materials and methods

Test item: GLOB1310aH (Aclonifen + Flufenacet 540 + 60 SC); Batch No.: GLO-20F-2306A

Content of active substance (a.s.):	nominal	analysed
Aclonifen:	540 g/L	543.7 g/L
Flufenacet:	60 g/L	61.55 g/L

Reference item: Dimethoate 400 EC was tested parallel to test item (analysed content of 411.20 ± 3.47 g/L)

Test species: Honeybee – *Apis mellifera* L. subspecies *iberiensis* E. (Hymenoptera, Apoidea): worker bees of a healthy and queen-right colony; female, adult worker bees (normally, at an age of 3 to 5 weeks) were collected in the morning before use; apiary: BioChem AGROLOGÍA S.L.U., Finca La Dehesilla, Ctra. A-362, Km. 4.7, P.O. Box 254, 41710 Utrera (Seville), Spain

Guideline(s): OECD 213 (1998), OECD 214 (1998)

Test design: Test item:

Contact test: 96-h; 2 control groups of deionised water, 1 % v/v tween solution; 5 dose rates of test item; 4 dose rates of the reference item; comprising 3 replicates per dose rate each of 10 bees, application volume: 4 µL/bee

Oral test: 96-h; 1 control group of 50 % w/v sucrose solution; 5 dose rates of test item; 4 dose rates of the reference item; comprising 3 replicates per dose rate each of 10 bees; application volume: 200 µL/cage by group feeding of 10 bees (corresponding to 20 µL/bee)

The mortality and the behaviour were assessed 4, 24, 48, 72, 96 hours after application f or the contact and oral test

Endpoints: Mortality, behavioural impairments

Dose rates [product/bee]

Test item:

Contact test: 2000, 1200, 720, 432, 259 µg product/bee
Oral test (offered): 2000, 1400, 980, 686, 480 µg product/bee
Oral test (consumed): 2000, 1400, 980, 686, 480 µg product/bee*

Dose rates [total a.s./bee] based on sum of nominal content of a.s.

Test item

Contact test: 977, 586, 352, 211, 126 µg a.s./bee
Oral test (offered): 977, 684, 479, 335, 236 µg a.s./bee
Oral test (consumed): 977, 684, 479, 335, 236 µg a.s./bee*
* based on the actual food uptake

Test conditions: Temperature: 24.1 – 26.8 °C (contact and oral)

Relative humidity: 54.1 – 69.2 % (contact and oral)

Illumination: constant darkness throughout the test (diffuse artificial light only during handling and assessments)

Food: 50 % (w/v) sucrose solution (after application ad libitum)

Statistics: Statistical program used: ToxRat Professional 3.3.0 (2018)

Calculation of LD50 values:

Test item:

Contact: no LD50-calculation due to low mortality
Oral: Probit analysis (linear maximum likelihood regression)

Reference item:

Contact: Probit analysis (linear maximum likelihood regression)
Oral: Probit analysis (linear maximum likelihood regression)

Statistical significance of mortality values:

Test item: Fisher's Exact Binomial Test with Bonferroni Correction ($\alpha = 0.05$)
Reference item: Fisher's Exact Binominal Test with Bonferroni Correction (α =

0.05)
Validity criteria Control mortality (48 h): ≤ 10 %
LD50 – value of the reference (24 h): 0.10 – 0.30 µg a.s./bee (contact)
0.10 – 0.35 µg a.s./bee (oral)
Experimental phase: 01 – 05 February 2021

Results and discussions

Contact test

After 48 hours, the control groups either treated with deionised water or 1 % tween solution demonstrated no mortality. In the test item treatment group, statistically significant mortality of 26.7 % was observed after thoracic application of 2000 µg GLOB1310aH/bee, respectively, after 48 hours.

Due to a significant increase of the bee mortality between the 24 hour and 48 hour assessments, the contact toxicity test was extended up to 96 hours. After 96 hours, the control groups either treated with deionised water or 1 % tween solution demonstrated no mortality. In the test item treatment group, statistically significant mortality of 36.7 and 20.0 % was observed after thoracic application of 2000 and 1200 µg GLOB1310aH/bee, respectively, after 48 hours.

Oral test

After 48 hours, the control group fed 50 % sucrose solution demonstrated no mortality. In the test item treatment group, statistically significant mortality of 60.0, 20.0 and 30.0 % was observed after oral consumption of 2000, 1400 and 980 µg GLOB1310aH/bee, respectively, after 48 hours.

Due to a significant increase of the bee mortality between the 24 hour and 48 hour assessments, the oral toxicity test was extended up to 96 hours. After 96 hours, the control group fed 50 % sucrose solution demonstrated no mortality. In the test item treatment group, statistically significant mortality of 70.0, 33.3 and 33.3 % was observed after oral consumption of 2000, 1400 and 980 µg GLOB1310aH/bee, respectively, after 96 hours.

LD_{50} values	Contact toxicity test			
	24h	48h	72h	96h
LD_{50} (µg product/bee)	>2000	>2000	>2000	>2000
LD_{50} (µg product/bee)*	>977	>977	>977	>977
LD_{50} values	Oral toxicity test**			
	24h	48h	72h	96h
LD_{50} (µg product/bee) (95% confidence limits)	>2000	>2000	1859 (1460-2925)	1676 (1382-2382)
LD_{50} (µg product/bee)* (95% confidence limits)	>977	>977	908 (713-1429)	819 (665-1164)

*Based on Sum of analysed content of Aclonifen and Flufenacet
**Oral dose rates based on actual consumed doses

Conclusion

The acute contact and oral toxicity of GLOB1310aH was tested on honeybees under laboratory conditions over 96 hours.

The contact 48 h LD50 was > 2000 µg GLOB1310aH/bee (corresponding to > 977 µg total a.s./bee) and contact 96 h LD50 was > 2000 µg GLOB1310aH/bee (corresponding to > 977 µg total a.s./bee). The oral 48 h LD50 was > 2000 µg GLOB1310aH/bee (corresponding to > 977 µg total a.s./bee) and oral 96 h LD50 was 1676 µg GLOB1310aH/bee (corresponding to 819 µg total a.s./bee).

A 2.3.1.1.2 KCP 10.3.1.1.2 Acute contact toxicity to bumblebees

Comments of zRMS:	The study was conducted to OECD guidelines 246 and 247 and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.1.1.2
Report	Acute toxicity of GLOB1310aH to the bumblebee *Bombus terrestris* L. under laboratory conditions. Amsel, K., Study No. 21 48 BBA 0001
Guideline(s):	Yes (OECD TG 246 and 247)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

SUMMARY

CONTACT TOXICITY TEST

In the contact toxicity test, young adult worker bumblebees (*Bombus terrestris* L.) were exposed to GLOB1310aH. The toxicity of the test item was determined at one dose rate of 818.8 µg product/bumblebee (equivalent to 400.0 µg total a.s./bumblebee).
Additionally, bumblebees were treated with Dimethoate EC 400 as reference item at a dose rate of 10.0 µg a.s./bumblebee and furthermore with deionised water and 0.5% (v/v) TritonX solution as controls.

I. MATERIAL AND METHODS

Test item:	GLOB1310aH, Batch No.: GLO-20F-2306A, Density: 1.2282 g/mL		
	Content of active substance:	nominal	analysed
	Aclonifen:	540 g/L	543.7 g/L
	Flufenacet:	60 g/L	61.55 g/L
Test species:	*Bombus terrestris* L. (bumblebee), adult worker bumblebees derived from queen right bumblebee hives; source: Biobest Belgium N.V., Ilse Velden 18, 2260 Westerlo, Belgium delivered: Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany; collected from 5 bumblebee hives under red light in the evening prior to testing.		
Test design:	In a 48 hours test, adults of *Bombus terrestris* were exposed to 1 dose rate of GLOB1310aH in an appropriate carrier (0.5% (v/v) TritonX solution) placed on the dorsal bumblebee thorax. In total, 3 treatment groups were set up: 2 control groups, 1 dose rate of the test item and 1 dose rate of the reference item with 50 replicates per dose for controls and test item and 30 replicates for reference item and one bumblebee per replicate, respectively. Assessments of bumblebee mortality and behavioural effects were done after 4, 24 and 48 hours.		
Endpoints:	Mortality, behavioural abnormalities		
Reference item:	Dimethoate EC 400 (analysed content of dimethoate: 411.20 g/L)		
Treatments:	Water control (deionised water)		

TritonX control (0.5% (v/v) TritonX solution)
Test item at dose rate of:
818.8 µg product/bumblebee (equivalent to 400.0 µg total a.s./bumblebee)
Reference item at a dose rate of:
10.0 µg dimethoate/bumblebee

Test conditions:	Temperature:	23.5 °C – 24.4 °C; relative humidity: 47% - 76%
	Photoperiod:	24 h darkness
	Food:	50% (w/v) sucrose solution

II. RESULTS AND DISCUSSION

After 48 hours of contact exposure, no mortality occurred in the control groups treated with deionised water and 0.5% (v/v) TritonX solution. In the test item treatment, no mortality was observed at the dose rate of 818.8 µg product/bumblebee after 48 hours. No behavioural effects appeared at the tested dose rate of GLOB1310aH during the contact toxicity test.

The results of the contact test are summarised in the following Tables I and II.

Table I: Contact toxicity of GLOB1310aH to *Bombus terrestris*

Treatment group [dosage unit]	**Dosage** applied	**Mean mortality [%]**	
		24 h	**48 h**
Control	Water	0.0	0.0
	0.5% (v/v) TritonX	0.0	0.0
GLOB1310aH [µg product/bumblebee]	818.8	0.0	0.0

Mortality in the reference item treatment in the contact test was 100.0% after 48 hours.

Table II: Contact toxicity of GLOB1310aH to *Bombus terrestris*, LD_{50} / NOED values

	Endpoint	24 h	48 h
GLOB1310aH	LD_{50} [µg product/bumblebee] LD_{50} [µg a.s./bumblebee]	**> 818.8** **> 400.0**	**> 818.8** **> 400.0**
	NOED [µg product/bumblebee] NOED [µg a.s./bumblebee]	**≥ 818.8** **≥ 400.0**	**≥ 818.8** **≥ 400.0**

III. CONCLUSION

In the acute contact toxicity test with GLOB1310aH, the resulting LD_{50} after 48 hours was > 818.8 µg product/bumblebee (equivalent to > 400.0 µg total a.s./bumblebee) and the NOED was ≥ 818.8 µg product/bumblebee (equivalent to ≥ 400.0 µg total a.s./bumblebee).

ORAL TOXICITY TEST

In the oral toxicity test, young adult worker bumblebees (*Bombus terrestris* L.) were exposed to GLOB1310aH. The toxicity of the test item was determined at dose rates of 921.6, 682.0, 504.7, 373.5 and 276.4 µg product/bumblebee (equivalent to 450.2, 333.2, 246.5, 182.4 and 135.0 µg total a.s./bumblebee). The resulting oral uptake was 716.9, 479.4, 401.5, 283.9 and 213.9 µg product/bumblebee (equivalent to 350.2, 234.2, 196.1, 138.7 and 104.5 µg total

a.s./bumblebee).
Additionally, bumblebees were treated with Dimethoate EC 400 as reference item at a dose rate of 1.39 µg consumed dimethoate/bumblebee and furthermore with a 50% (w/v) sucrose solution as control.

I. MATERIAL AND METHODS

Test item:	GLOB1310aH, Batch No.: GLO-20F-2306A, Density: 1.2282 g/mL		
	Content of active substance:	nominal	analysed
	Aclonifen:	540 g/L	543.7 g/L
	Flufenacet:	60 g/L	61.55 g/L
Test species:	*Bombus terrestris* L. (bumblebee), adult worker bumblebees derived from queen right bumblebee hives; source: Biobest Belgium N.V., Ilse Velden 18, 2260 Westerlo, Belgium delivered: Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany; collected from 5 bumblebee hives under red light in the evening prior to testing with a starvation periode of 4 hours before test begin.		
Test design:	In a 48 hours test, adults of *Bombus terrestris* L. were exposed to 5 dose rates of GLOB1310aH in treated food (50% (w/v) sucrose solution). In total, 3 treatment groups were set up: 1 control group, 5 dose rates of the test item and 1 dose rate of the reference item with 30 replicates per dose and one bumblebee per replicate, respectively. Assessments of bumblebee mortality and behavioural effects were done after 4, 24 and 48 hours.		
Endpoints:	Mortality, behavioural abnormalities		
Reference item:	Dimethoate EC 400 (analysed content of dimethoate: 411.20 g/L)		
Treatments:	Sucrose control (50% (w/v) sucrose solution) Test item at dose rates of: 921.6, 682.0, 504.7, 373.5 and 276.4 µg product/bumblebee (equivalent to 450.2, 333.2, 246.5, 182.4 and 135.0 µg total a.s./bumblebee) actual uptake: 716.9, 479.4, 401.5, 283.9 and 213.9 µg product/bumblebee (equivalent to 350.2, 234.2, 196.1, 138.7 and 104.5 µg total a.s./bumblebee) Reference item at a dose rate of: 1.51 µg dimethoate/bumblebee (actual uptake: 1.39 µg dimethoate/bumblebee)		
Test conditions:	Temperature:	23.5 °C – 24.4 °C; relative humidity: 47% - 76%	
	Photoperiod:	24 h darkness	
	Food:	50% (w/v) sucrose solution	

II. RESULTS AND DISCUSSION

In the oral toxicity test, no mortality occurred in the control group fed with 50% (w/v) sucrose solution. In the test item treatment, no mortality occurred in the dose rates of 716.9, 479.4, 401.5, 283.9 and 213.9 µg consumed product/bumblebee after 48 hours. No behavioural effects appeared at all tested dose rates of GLOB1310aH during the oral toxicity test.

The results of the oral test are summarised in the following Tables III and IV.

Table III: Oral toxicity of GLOB1310aH to *Bombus terrestris*

Treatment group [dosage unit]	**Dosage** consumed	**Mean mortality [%]**	
		24 hours	**48 hours**
Control	Sucrose solution	0.0	0.0
GLOB1310aH [µg product/ bumblebee]	716.9	0.0	0.0
	479.4	0.0	0.0
	401.5	0.0	0.0
	283.9	0.0	0.0
	213.9	0.0	0.0

Mortality in the reference item treatment in the oral test was 82.1% after 48 hours.

Table IV: Oral toxicity of GLOB1310aH to *Bombus terrestris*, LD_{50} / NOED values

	Endpoint[1]	24 h	48 h
GLOB1310aH	LD_{50} [µg product/bumblebee] LD_{50} [µg total a.s./bumblebee]	**> 716.9** **> 350.2**	**> 716.9** **> 350.2**
	NOED [µg product/bumblebee] NOED [µg total a.s./bumblebee]	**≥ 716.9** **≥ 350.2**	**≥ 716.9** **≥ 350.2**

[1] based on consumed values

III. CONCLUSION

In the acute oral toxicity test with GLOB1310aH, the resulting LD_{50} after 48 hours was > 716.9 µg consumed product/bumblebee (> 350.2 µg consumed total a.s./bumblebee) and the NOED after 48 hours was ≥ 716.9 µg consumed product/bumblebee (≥ 350.2 µg consumed total a.s./bumblebee).

A 2.3.1.2 KCP 10.3.1.2. Chronic toxicity to bees

Comments of zRMS:	The study was conducted to OECD guidelines 245 and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.1.2
Report	Chronic toxicity to the honey bee *Apis mellifera* L. under laboratory conditions. Dressler, K., 2021. Study No. 21 48 BAC 0004
Guideline(s):	Yes (OECD TG 245 (2017))
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

Materials and methods

Test item: GLOB1310aH (Aclonifen + Flufenacet 540 + 60 SC); Batch No.: GLO-20F-2306A

Content of active substance (a.s.):	nominal	analysed
Aclonifen:	540 g/L	543.7 g/L
Flufenacet:	60 g/L	61.55 g/L

Reference item: Danadim® Progress, batch no.: 10214034

Content of active ingredient:	nominal	analysed
Dimethoate:	400 g/L	411.20 g/L

Density (at 20 °C): 1.069 g/mL

Test species: *Apis mellifera* L. subspecies *iberiensis* (honey bee), not older than 2 days and derived from healthy and queen-right colonies; source: BioChem AGROLOGÍA S.L.U., Finca La Dehesilla, Ctra. A-362, Km. 4.7, P.O. Box 254, 41710 Utrera (Sevilla), Spain

Guideline: OECD TG 245 (2017)

Deviations from the guideline: none

Test design: In a 10-day chronic toxicity feeding test, young adults of *Apis mellifera* L. (not older than 2 days) were continuously exposed to GLOB1310aH diluted in the bee food (50% (w/v) sucrose solution + 0.1% (w/v) xanthan).

The following treatment groups were set up: 7 doses of the test item, 1 untreated control group AC fed with 50% (w/v) sucrose solution, 1 untreated control group BC fed with 50% (w/v) sucrose solution + 0.1% (w/v) xanthan and 1 dose of the reference item. For each treatment group, 3 replicates per dose and 10 bees per replicate were used.

All feeding solutions were freshly prepared every day and provided ad libitum (minimum quantity of 2 mL). Assessments of bee mortality, food consumption and behavioural abnormalities were conducted daily.

In the analytical phase of the study, the concentration of the active ingredients Aclonifen and Flufenacet in the highest and lowest test item feeding solution applied on each day of application was determined.

Endpoints: Mortality, behavioural abnormalities

Test concentrations: Control group AC:

untreated food (50% (w/v) sucrose solution)

Control group BC:

untreated food (50% (w/v) sucrose solution + 0.1% (w/v) xanthan)

Test item group:

treated food at nominal doses of 200, 95.2, 45.4, 21.6, 10.3, 4.90 and 2.33 µg product/bee/day (equivalent to 87.9, 41.9, 19.9, 9.50, 4.52, 2.15 and 1.02 µg Aclonifen/bee/day*, 9.77, 4.65, 2.22, 1.06, 0.502, 0.239 and 0.114 µg Flufenacet/bee/day*) corresponding to concentrations of 5092, 2425, 1155, 550, 262, 125, 59.3 mg product/kg food (equivalent to 2239, 1066, 508, 242, 115, 54.9 and 26.1 mg Aclonifen/kg food* 249, 118, 56.4, 26.9, 12.8, 6.10 and 2.90 mg Flufenacet/kg food*)

Effectively consumed doses: 193, 80.4, 43.7, 22.6, 8.99, 4.11 and 1.95 µg product/bee/day (equivalent to 85.0, 35.3, 19.2, 9.92, 3.95, 1.81 and 0.859 µg Aclonifen/bee/day*, 9.44, 3.93, 2.13, 1.10, 0.439, 0.201 and 0.0954 µg Flufenacet/bee/day*)

* calculations were based on the nominal content of active ingredients and the product density (according to the Certificate of Analysis of 16 Dec 2020, see Appendix 8)

Reference item group: treated food at a nominal dose of 27.3 ng dimethoate/bee/day (corresponding to a concentration of 0.694 mg dimethoate/kg food)

Test conditions: Temperature: 33.2 – 33.9 °C

Relative humidity: 60.1 – 66.8%

Photoperiod: darkness (diffuse artificial light only during assessments and exchange of feeders)

Food: 50% (w/v) sucrose solution

Statistics: Statistical software used: ToxRat Professional 3.3.0 (2018).

Step-down Cochran-Armitage Test Procedure for mortality data and determination of

NOEDD/NOEC (α = 0.05, one-sided greater). Since the obtained mortalities did not reach 50%, the LDD50 and LC50 values could not be calculated. They were estimated from the generated raw data. Probit analysis using linear maximum likelihood regression for the calculation of LDD20/10 and LC20/10 values along with their 95% confidence limits.

Dates of work: Experimental starting date (biological phase): 16 Mar 2021
Experimental completion date (biological phase): 26 Mar 2021
Experimental starting date (analytical phase): 03 May 2021
Experimental completion date (analytical phase): 04 May 2021

Results and discussions

After 10 days of continuous exposure, a mean mortality of 0.0% was observed in both the blank control group AC and viscosifier control group BC. Finally, in the reference item group, a mean mortality of 100% was recorded. Therefore, all validity criteria for the study were met.

Taking into account the actual food uptake and evaporated amount of feeding solution, the bees effectively consumed doses of 193, 80.4, 43.7, 22.6, 8.99, 4.11 and 1.95 µg product/bee/day which resulted in mortalities of 43.3, 16.7, 3.3, 0.0, 0.0, 0.0 and 0.0% after 10 days, respectively. The obtained mortalities in the two highest test item doses (193 and 80.4 µg consumed product/bee/day) were statistically significantly increased compared to the viscosifier control group BC (Step-down Cochran-Armitage Test Procedure, α = 0.05, one-sided greater).

During the course of the test, behavioural abnormalities were observed in the highest test item dose (193 µg consumed product/bee/day). Several bees were observed as being affected (uncoordinated movements) from day 6 onwards. On the final assessment day (day 10), 13 out of 17 bees were observed as being affected (uncoordinated movements). Another 2 bees were observed as being moribund. No other behavioural abnormalities were observed in any test item treatment group on any other assessment day.

Results are summarised in Tables I and II.

Table I. Mean mortality and behaviour of bees in the chronic toxicity feeding test after 10 days

treatment group	treat-ment group ID	daily dose nominal [µg product/bee/day]	daily dose consumed[1] [µg product/bee/day]	concentration [mg product/kg food]	after 10 days: mean mortality absolute [%]	after 10 days: mean mortality corrected [%]	after 10 days: number of bees showing behavioural abnormalities[2]
blank control	AC	--	--	--	0.0	--	0 out of 30
viscosifier control	BC	--	--	--	0.0	--	0 out of 30
test item	AT	200	193	5092	43.3*	--	15 out of 17
	BT	95.2	80.4	2425	16.7*	--	0 out of 25
	CT	45.4	43.7	1155	3.3	--	0 out of 29
	DT	21.6	22.6	550	0.0	--	0 out of 30
	ET	10.3	8.99	262	0.0	--	0 out of 30
	FT	4.90	4.11	125	0.0	--	0 out of 30
	GT	2.33	1.95	59.3	0.0	--	0 out of 30
		[ng a.i./bee/day]		[mg a.i./kg food]			
reference item	AR	27.3	22.3	0.694	100	--	--

Results are averages based on 3 replicates, containing 10 bees each. Calculations were performed with non-rounded values.
corrected: corrected mortality (according to SCHNEIDER-ORELLI 1947); Due to 0.0% mortality in both control groups, correction is not required.

* statistically significant difference in pairwise comparison between treatment and untreated viscosifier control group BC (Step-down Cochran-Armitage Test Procedure, $\alpha = 0.05$, one-sided greater)

[1] taking into account the actual food uptake and evaporation

[2] number of bees showing behavioural abnormalities referring to the number of remaining bees

Table II. Statistical endpoints

	endpoints	after 10 days
test item doses	LDD_{50} [µg consumed product/bee/day[1]][2]	> 193
	LDD_{20} [µg consumed product/bee/day[1]][3]	101 (72.7 – 137)
	LDD_{10} [µg consumed product/bee/day[1]][3]	68.4 (41.2 – 91.6)
	NOEDD [µg consumed product/bee/day[1]][4]	43.7
	LDD_{50} [µg consumed Aclonifen/bee/day[1]][2]	> 84.9
	LDD_{20} [µg consumed Aclonifen/bee/day[1]][3]	44.5 (32.0 – 60.2)
	LDD_{10} [µg consumed Aclonifen/bee/day[1]][3]	30.1 (18.1 – 40.3)
	NOEDD [µg consumed Aclonifen/bee/day[1]][4]	19.2
	LDD_{50} [µg consumed Flufenacet/bee/day[1]][2]	> 9.43
	LDD_{20} [µg consumed Flufenacet/bee/day[1]][3]	4.95 (3.55 – 6.69)
	LDD_{10} [µg consumed Flufenacet/bee/day[1]][3]	3.34 (2.01 – 4.48)
	NOEDD [µg consumed Flufenacet/bee/day[1]][4]	2.13
test item concentrations	LC_{50} [mg product/kg food][2]	> 5092
	LC_{20} [mg product/kg food][3]	2799 (1992 – 3733)
	LC_{10} [mg product/kg food][3]	1918 (1112 – 2556)
	NOEC [mg product/kg food][4]	1155
	LC_{50} [mg Aclonifen/kg food][2]	> 2239
	LC_{20} [mg Aclonifen/kg food][3]	1231 (876 – 1641)
	LC_{10} [mg Aclonifen/kg food][3]	843 (489 – 1124)
	NOEC [mg Aclonifen/kg food][4]	508
	LC_{50} [mg Flufenacet/kg food][2]	> 249
	LC_{20} [mg Flufenacet/kg food][3]	137 (97.3 – 182)
	LC_{10} [mg Flufenacet/kg food][3]	93.7 (54.3 – 125)
	NOEC [mg Flufenacet/kg food][4]	56.4

Calculations were performed with non-rounded values.
[1] taking into account the actual food uptake and evaporation
[2] medium lethal dietary doses/concentrations were estimated from the generated raw data
[3] lethal dietary doses/concentrations (95%-cl lower – upper) were calculated using Probit analysis (linear max. likelihood regression)
[4] no observed effect dietary doses/concentrations were determined using Step-down Cochran-Armitage Test Procedure ($\alpha = 0.05$, one-sided greater)

In the test item treatment group, the overall mean daily food consumption ranged between 32.9 and 41.0 mg feeding solution/bee/day which corresponds to 83.8% and 104.4% of the expected daily amount. In blank control group AC, the bees consumed on average 38.6 mg feeding solution/bee/day (corresponding to 98.4% of the expected daily amount). In viscosifier control group BC, the bees consumed on average 38.8 mg feeding solution/bee/day (corresponding to 98.9% of the expected daily amount).

The daily mean evaporation of 50% (w/v) sucrose solution ranged between 49.0 and 65.0 mg per cage. The daily mean evaporation of 50% (w/v) sucrose solution + 0.1% (w/v) xanthan ranged between 46.0 and 57.7 mg per cage. The food consumption per cage was corrected by subtracting the respective mean evaporation figure of the respective day of application.

The recovery rates of the active ingredients Aclonifen and Flufenacet in the analysed samples of the test item feeding solutions were between ± 20% of the nominal concentrations. Therefore, the concentrations of active ingredients in the applied test item feeding solutions were verified and endpoints have been based on nominal concentrations. Furthermore, no residues of the active ingredients were found in the control samples, i.e., the concentrations of active ingredients were below 30% of the LOQ (limit of quantification).

Conclusion

The chronic oral toxicity of GLOB1310aH to young adult honey bees (*Apis mellifera* L.) was investigated in a 10-day chronic, dose-response feeding study under laboratory conditions.

Since the obtained mortalities did not reach 50%, the LDD50 was estimated to be greater than 193 µg consumed product/bee/day and the LC50 was estimated to be greater than 5092 mg product/ kg food. The LDD20 was calculated to be 101 µg consumed product/bee/day and the LC20 was calculated to be 2799 mg product/kg food. The LDD10 was calculated to be 68.4 µg consumed product/bee/day and the LC10 was calculated to be 1918 mg product/kg food.

The NOEDD was determined to be 43.7 µg consumed product/bee/day, corresponding to a NOEC of 1155 mg product/kg food

A 2.3.1.3 KCP 10.3.1.3 Effects on honey bee development and other honey bee life stages

Comments of zRMS:	The study was conducted to OECD guidelines 239 and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.1.3
Report	GLOB1310aH - Repeated exposure of honey bee (*Apis mellifera* L.) larvae under laboratory conditions. Hänsel, M., 2021. Study No. 21 48 BLC 0003
Guideline(s):	Yes (OECD 239 (2016))
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

SUMMARY

In a test under laboratory conditions, honey bee larvae (*Apis mellifera* L.) were repeatedly exposed to GLOB1310aH. The toxicity of the test item was determined at cumulative doses of 59.2, 29.6, 14.8, 7.4 and 3.7 µg product/larva. The concentrations of test item in the diets were 373.9, 187.0, 93.5, 46.7 and 23.4 mg product/kg food. Additionally, honey bee larvae were treated with Dimethoate tech. as reference item at a total dose of 7.6 µg dimethoate/larva or with an untreated diet as control.

III. MATERIAL AND METHODS

Test item:	GLOB1310aH, Batch No.: GLO-20F-2306A
Reference item:	Dimethoate tech. (analysed purity: 98.8% ± 0.5%)
Test species:	*Honey bee – Apis mellifera iberiensis* Engel (Hymenoptera, Apoidea): first instar larvae; derived from three healthy and queen-right colonies; source: BioChem AGROLOGÍA S.L.U., Utrera (Seville), Spain
Test design:	One day old honey bee larvae (D1) of *Apis mellifera* L. were transferred from brood combs to polystyrene grafting cells in 48-well cell culture plates 2 days be-

fore start of the treatment. On 4 successive days (D3 to D6) the larvae were repeatedly exposed to GLOB1310aH diluted in the larval food (aqueous sugar solution mixed with royal jelly). After the applications, no additional feedings of the larvae took place. In total, 7 treatment groups were set up: 5 doses of the test item, one untreated control group and 1 dose of the reference item with 3 replicates per dose and 12 larvae per replicate, each. Assessments of larval mortality were performed on D4, D5, D6, D7 and D8. Additionally, other observations such as small body size or large quantities of remaining food on D8 were noted. Pupal mortality was assessed on D15 and emergence of adults was evaluated on D22.
In an analytical phase of the study the concentration of the active ingredients in the test item final diets and in the control was determined.

Endpoints: Successful adult emergence, mortality, qualitative observations: *e.g.* body size, remaining food

Test concentrations:

Controls:	AC	untreated diet B/C (aqueous sugar solution + royal jelly)
Test item:	AT	treated diet B/C at a concentration of 373.9 mg product/kg food
	BT	treated diet B/C at a concentration of 187.0 mg product/kg food
	CT	treated diet B/C at a concentration of 93.5 mg product/kg food
	DT	treated diet B/C at a concentration of 46.7 mg product/kg food
	ET	treated diet B/C at a concentration of 23.4 mg product/kg food
Reference:	AR	treated diet B/C at a concentration of 48 mg a.i./kg food

Test conditions:

Temperature:	34.0 °C – 34.5 °C
Relative humidity:	D1 - D8: 92.4 – 100.0%; D8-D15: 80.3 – 84.9%; D15-D22: 55.1 – 69.5%
Photoperiod:	Darkness (except during assessments)
Food:	aqueous sugar solution with royal jelly

Statistics: Descriptive statistics, Step-down Cochran-Armitage Test procedure (one-sided greater, alpha = 0.05) for determination of NOED/NOEC, ED/$EC_{10/20}$ values were determined by Weibull analysis using linear max. likelihood regression. Due the fact that the mortality was below 50% even in the highest test item treatment group (59.2 µg product/larva) the ED/EC_{50} was estimated to be > 59.2 µg product/larva.

IV. RESULTS AND DISCUSSION

The results are summarised below.

Toxicity of GLOB1310aH to larvae of *Apis mellifera* L. after repeated exposure

Treatment group	Treatment ID	Dose	Concentration	On D8			On D15		On D22		
				Larval mortality D3 to D8		Mean OO	Pupal mortality D8-D15		Total mortality D3-D22		Adult emergence rate
				[%]		[%]	[%]		[%]		[%]
		[µg product/ larva]	[mg product/ kg food]	abs.	corr.		abs.	corr.	abs.	corr.	abs.
Control	AC	-	-	0.0	-	0.0	13.9	-	22.2	-	77.8
Test item	AT	59.2	373.9	16.7	16.7	0.0	10.0	0.0	44.4	28.6	55.6*
	BT	29.6	187.0	16.7	16.7	0.0	9.4	0.0	27.8	7.1	72.2

	CT	14.8	93.5	2.8	2.8	0.0	19.9	7.0	27.8	7.1	72.2
	DT	7.4	46.7	2.8	2.8	0.0	16.9	3.5	25.0	3.6	75.0
	ET	3.7	23.4	0.0	0.0	0.0	16.7	3.2	22.2	0.0	77.8
Reference item	AR	[µg a.i./ larva] 7.6	[mg a.i./ kg food] 48	75.0	75.0	0.0	66.7	61.3	94.4	92.9	5.6

Treatment	Endpoint: Successful adult emergence	Up to D22
Test item doses	ED_{50} [µg product/larva] [2]	> 59.2
	ED_{20} [µg product/larva] [2]	46.015 (30.980 – 68.345)
	ED_{10} [µg product/larva] [2]	25.613 (16.742 – 39.185)
	NOED [µg product/larva] [1]	29.6
Test item concentrations	EC_{50} [mg product/kg food] [2]	> 373.9
	EC_{20} [mg product/kg food] [2]	290.651 (195.682 – 431.709)
	EC_{10} [mg product/kg food] [2]	161.783 (105.749 – 247.509)
	NOEC [mg product/kg food] [1]	187.0

Results are averages based on 3 replicates, containing 12 larvae each; see Appendix 4 for details
corr.: corrected mortality (according to SCHNEIDER-ORELLI 1947): test and reference item treated goups were corrected by AC; negative values were set to "0"; calculations were performed with non-rounded values; CL: confidence limit; abs.: absolute mortality as counted from the results; OO: Other observations (*e.g.* remaining food); Average% of pupal mortality: Sum of dead larvae between D8 and D15 / Sum of living larvae on D8 x 100% (replicate wise)
* Statistically significantly different to the control (Step-down Cochran-Armitage Test procedure; alpha=0.05; one sided greater)
[1] Step-down Cochran-Armitage Test procedure; alpha=0.05; one sided greater
[2] Weibull analysis using linear max. likelihood regression

On D8, a larval mortality of 0.0% was observed in the control (AC). Pupal mortality (between D8 and D15) was 13.9% in the control. The control group showed a total mortality of 22.2% on D22. In the test item treated groups, larval mortalities ranged between 0.0 and 16.7% on D8. Pupal mortalities (D8-D15) ranged between 9.4 and 19.9% in the test item treatment groups. Total mortalities ranged between 22.2 and 44.4% on D22. Mortality in the reference item treated group (AR) was above 50% across all replicates on D8, being 75.0%.

On D8, none of the remaining larvae treated with test item, were observed to have food left and/or a smaller body size.

In the final assessment on D22, an adult emergence rate of 77.8% was determined for the honey bees in the control group (AC). In the test item treated groups, the adult honey bees emerged at rates of: 55.6%, 72.2%, 72,2%, 75.0% and 77.8% following an application of 59.2, 29.6, 14.8, 7.4 and 3.7 µg product/larva, respectively, during the larval stages. On D22, larvae treated with 59.2 µg product/larva showed emergence rates, which were statistically significantly decreased if compared to the control.

The recoveries of active ingredient in the test item final diets AT to ET ranged between 90.7% and 100.7%. No test item was detected in the control specimen.

Because control mortality was ≤ 15% on D8, cumulative mortality in the reference item treatment group was ≥ 50% on D8 and adult emergence in the control was ≥ 70% on D22, the study can be regarded as valid

III. CONCLUSION

In a repeated exposure larval toxicity study with GLOB1310aH, the ED_{50} (adult emergence up to D22) was estimated to be > 59.2 µg product/larva, which is equivalent to an EC_{50} of > 373.3 mg product/kg food. The ED_{20} was determined to be 46.0 µg product/larva, which is equivalent to an EC_{20} of 290.7 mg

product/kg food. The ED_{10} was determined to be 25.6 µg product/larva, which is equivalent to an EC_{10} of 161.8 mg product/kg food.
The NOED was 29.6 µg product/larva and the corresponding NOEC was 187.0 mg product/kg food.

A 2.3.1.4 KCP 10.3.1.4 Sub-lethal effects

A 2.3.1.5 KCP 10.3.1.5 Cage and tunnel tests

A 2.3.1.6 KCP 10.3.1.6 Field tests with honeybees

A 2.3.2 KCP 10.3.2 Effects on arthropods other than bees

Comments of zRMS:	The study was conducted to HEIMBACH *et al.* 2000 guideline and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.2
Report	Effects of GLOB1310aH on the carabid beetle *Poecilus cupreus* L. in an extended laboratory test (Limit-test). Röhlig, U., 2021a. Study No. 21 48 NLE 0001
Guideline(s):	Yes (IOBC (HEIMBACH *et al.* 2000))
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

Executive summary

An extended laboratory study was carried out to determine the effects of the test item GLOB1310aH on the carabid beetle *Poecilus cupreus* L. (Coleoptera: Carabidae). For determination of the mortality adult beetles were exposed to fresh dried spray residues of the test item applied onto sandy soil (LUFA 2.1). Effects on mortality were assessed by the number of surviving beetles, additionally behavioural impacts (food uptake) were assessed.

The study encompassed 3 treatment groups (1 test item rate, control, reference item), each with 5 replicates. Three females and three males per replicate were exposed to dried residues of GLOB1310aH sprayed onto sandy soil at a rate of 2 L product/ha in 400 L/ha. Additional test units were treated with deionised water as control and with DANADIM PROGRESS (active substance 411.2 g Dimethoate/L) as reference item. Endpoints of the study were mortality and additionally effects on the food uptake.

After 14 days, a mortality of 0 % was observed in the water-treated control. In the test item treatment, mortality was also 0 %. This resulted in a corrected mortality rate of 0 %. No statistically significant effects on mortality were observed at the tested rate (FISHER'S Exact Binomial test, $\alpha = 0.05$).

The food uptake (mean number of consumed fly pupae per surviving beetle during the total study period) was 11.00 fly pupae in the test item treatment group, in comparison to the control with 10.37 fly pupae. No statistically significant effects on food uptake were determined (STUDENT-t-Test, $\alpha = 0.05$) at the tested rate.

In an extended laboratory study, no effects on mortality and food uptake for *Poecilus cupreus* were observed, when GLOB1310aH was applied at a rate of 2 L product/ha in 400 L water/ha. The LR_{50} as well as the ER_{50} were estimated to be > 2 L product/ha. The NOER for mortality as well as for food uptake were estimated to be 2 L product/ha.

Materials & methods

Test item:	GLOB1310aH, batch No.: GLO-20F-2306A analysed content of a.i.: Aclonifen: 543.7 g/L (nominal 540 g/L) Flufenacet: 61.55 g/L density: 1.2282 g/mL
Test species:	Carabid beetle *Poecilus cupreus* L. adults (2-5 weeks old); source (in-house culture): in the laboratory of the test facility BioChem agrar GmbH
Test design:	Exposure of the adults was achieved via air-dried spray residues onto sandy soil (LUFA 2.1). Three treatment groups (1 test item rate, water-treated control, reference item) were set up with 5 replicates (consisting of 3 females and 3 males) per treatment. Mortality and behavioural assessments were carried out 2 hours, 1, 2, 4, 7, 11 and 14 days after application. Assessment of food uptake, *i.e.* number of consumed fly pupae, was made for the control and the test item groups on 1, 2, 4, 7, 11 and 14 days after application.
Endpoints:	Mortality: number of dead beetles, estimation of LR_{50}, NOER Food uptake: number of consumed fly pupae per surviving beetle, estimation of ER_{50} and NOER
Test rates:	Control (deionised water) Test item (GLOB1310aH): 2 L product/ha in 400 L water/ha Reference item (DANADIM PROGRESS) 2.25 L product/ha. in 400 L water/ha. The substances were sprayed onto sandy soil (LUFA 2.1) via laboratory spraying equipment and air dried afterwards.
Test conditions:	Temperature: 19 °C - 22 °C; relative humidity: 67 % - 72 % light-dark-cycle: 16 hours light : 8 hours dark; light intensity: 1030 lx Food: defrosted pupae of onion fly *Delia antiqua*
Statistics:	FISHER's Exact Binomial Test ($\alpha = 0.05$) for mortality (test item and reference item) STUDENT t-Test ($\alpha = 0.05$) for food uptake (test item and reference item)

Results & discussion

The results are summarised below.

After 14 days, a mortality of 0 % was observed in the water-treated control. In the test item treatment, mortality was also 0 %. This resulted in a corrected mortality rate of 0 %. No statistically significant effects on mortality were observed at the tested rate (FISHER'S Exact Binomial test, $\alpha = 0.05$).

The food uptake (mean number of consumed fly pupae per surviving beetle during the total study period) was 11.00 fly pupae in the test item treatment group, in comparison to the control with 10.37 fly pupae. No statistically significant effects on food uptake were determined (STUDENT-t-Test, $\alpha = 0.05$) at the tested rate.

Effects on the carabid beetle (*Poecilus cupreus)* exposed to fresh dry residues of GLOB1310aH in an extended laboratory test

Treatment	**Rate [1] [L product/ha]**	**Mortality [2] [%]**	**Corrected Mortality [3] [%]**	**Total number of consumed fly pupae**	**Food uptake [4] [mean number of consumed fly pupae/surviving beetle]**		**Effect on food uptake [5] [%]**
					during the total study period	per assessment day	
Control	-	0	-	311	10.37	1.73	-
Test item GLOB1310aH	2	0 (n.s.)	0	330	11.00	1.84 (n.s.)	-6.4
Reference item DANADIM PROGRESS	2.25	100*	100	17	0.57	0.29*	83.3

[1] Application rate in 400 L water/ha
[2] Mortality after 14 days of exposure to residues on sandy soil. The results for mortality in individual treatments were compared to that in the control using FISHER's Exact Binomial Test ($\alpha = 0.05$) for the test item and reference item.
[3] Corrected mortality according to ABBOTT (1925)
[4] Food uptake: mean number of consumed fly pupae/surviving beetle. The results for the test item treatment and control and the reference item treatment and control were compared by STUDENT-t-test, respectively ($\alpha = 0.05$).
[5] Change in mean number of consumed fly pupae per treatment group, relative to control. A positive value indicates a decrease and a negative indicates an increase relative to the control.
(n.s.) not statistically significant different compared to the control
* statistically significant different compared to the control

The reference item caused a mortality of 100 % of exposed beetles, resulting in a corrected mortality of 100 %.

Conclusion

In an extended laboratory study, no effects on mortality and food uptake for *Poecilus cupreus* were observed, when GLOB1310aH was applied at a rate of 2 L product/ha in 400 L water/ha. The LR_{50} as well as the ER_{50} were estimated to be > 2 L product/ha. The NOER for mortality as well as for food uptake were estimated to be 2 L product/ha.

Comments of zRMS:	The study was conducted to GRIMM *et al.* 2000 guideline and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.2
Report	Effects of GLOB1310aH on the rove beetle Aleochara bilineata GYLL. in an extended laboratory test (Limit-test). Röhlig, U., 2021b. Study No. 21 48 NKE 0001
Guideline(s):	Yes (IOBC (GRIMM *et al.* 2000))
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

Executive summary

An extended laboratory study was carried out to determine the effects of the test item GLOB1310aH on the rove beetle *Aleochara bilineata* (Coleoptera: Staphylinidae). For determination of the reproductive capacity adults were exposed to one application rate of GLOB1310aH sprayed onto sandy soil (LUFA 2.1). Effects on reproduction were assessed by the number of emerged beetles compared to the control group.

The study encompassed 3 treatment groups (1 test item rate, control, reference item), each with 4 replicates. 10 females and 10 males (10 pairs) per replicate were exposed to the test item sprayed onto sandy soil at an application rate of 2 L product/ha. Additional test units were treated with deionised water as control or with DANADIM PROGRESS (active substance 411.2 g Dimethoate/L) as reference item. The endpoint of the study was the reproductive capacity.

In the water-treated control the average number of hatched beetles of the F_1 generation was 473. In the test item treatment reproductive capacity was 447.
This resulted in an effect on reproduction of 5.4 % inhibition. No statistically significant differences compared to the control were observed at the test item treatment group (STUDENT-t-test, $\alpha = 0.05$), compared to the water-treated control.
The ER_{50} was estimated to be > 2 L product/ha.
The NOER (no observed effect rate) for reproductive capacity was determined to be ≥ 2 L product/ha.

In an extended laboratory study, the ER_{50} for *Aleochara bilineata* was estimated to be > 2 L product/ha. The NOER (no observed effect rate) for reproductive capacity was determined to be ≥ 2 L product/ha.

Materials & methods

Test item:	GLOB1310aH, batch No.: GLO-20F-2306A analysed content of a.i.: Aclonifen: 543.7 g/L (nominal 540 g/L) Flufenacet: 61.55 g/L (nominal 60 g/L) Density: 1.2282 g/mL
Test species:	Rove beetle *Aleochara bilineata* GYLL., adults (1-7 days old); source: reared in the laboratory of the test facility

Test design:

The test item rate, control and reference item were sprayed via a laboratory spray applicator (tracksprayer) on the soil surface. Exposure of the beetles was reached via air-dried residues on treated sandy soil (LUFA 2.1).

Three treatment groups (1 test item rate, water-treated control, reference item) were set up with 4 replicates (consisting of 10 females and 10 males (10 pairs) per treatment. On day 7, 14 and 21 approx. 500 pupae of *Delia antiqua* were buried in the sandy soil (LUFA 2.1) of each replicate to be parasitised by the larvae of the beetles. On day 28 the adults were separated from the soil and the sandy soil with the pupae was allowed to dry for seven days. On day 35 the pupae were removed from the soil by a sieve and transferred into a hatching unit. After hatching, the test endpoint reproductive capacity (average number of hatched beetles of the F_1 generation) was determined (daily assessments during 5 weeks).

Endpoint: Reproductive capacity (average number of hatched beetles of the F_1 generation)

Reference item: DANADIM PROGRESS
(Dimethoate 411.2 g/L, nominal: 400 g/L)

Test rates: Control (deionised water)
Test item: 2 L product/ha
with an application volume of 400 L/ha

The reference item was applied at a rate of 1.5 L/ha. All substances were applied in 400 L water/ha. The substances were sprayed onto sandy soil via laboratory spraying equipment and air dried afterwards.

Test conditions: Temperature: 19 °C - 22 °C;
relative humidity: 64 % - 75 %

light-dark-cycle: 16 hours light : 8 hours dark;

light intensity: 1850 lx

Food: *Chironomus* spp. larvae (thawed)

Statistics: STUDENT-t-test ($\alpha = 0.05$) for reproductive capacity (test item and reference item)

Results & discussion

In the water-treated control the average number of hatched beetles of the F_1 generation was 473. In the test item treatment reproductive capacity was 447.
This resulted in an effect on reproduction of 5.4 % inhibition. No statistically significant differences compared to the control were observed at the test item treatment group (STUDENT-t-test, $\alpha = 0.05$), compared to the water-treated control.
The ER_{50} was estimated to be > 2 L product/ha.
The NOER (no observed effect rate) for reproductive capacity was determined to be ≥ 2 L product/ha.

The results are summarised below.

Effects on reproductive capacity of the rove beetle (*Aleochara bilineata* GYLL.) exposed to GLOB1310aH in an extended laboratory test

Treatment	Rate[1] [L product/ha]	Reproduction [mean number of emerged beetles per replicate]	Reproduction [absolute number of emerged beetles per treatment group]	Effect on Reproduction[2] [%]
Control	-	473	1891	-
Test item	2	447 (n.s.)	1789	5.4
Reference item	1.5	12*	48	97.5

[1] Application rate in 400 L water/ha

[2] Effect on reproduction according to the following formula: (1-Pt/Pc) * 100% calculated on the absolute number of emerged beetles (positive values represent a decrease reproduction compared to the control)

n.s. statistically significantly different compared to the control: STUDENT-t-test, $\alpha = 0.05$ (test item)

* statistically significantly different compared to the control: STUDENT-t- test, $\alpha = 0.05$ (reference item)

No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.

Conclusion

In an extended laboratory study, the ER_{50} for *Aleochara bilineata* was estimated to be > 2 L product/ha. The NOER (no observed effect rate) for reproductive capacity was determined to be ≥ 2 L product/ha.

Comments of zRMS:	The study was conducted to Mead-Briggs *et al.* 2009 guidelines and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.2
Report	Effects of GLOB1310aH on the parasitic wasp *Aphidius rhopalosiphi* (DeStefani-Perez) in an extended laboratory test Röhlig, U., 2021c. Study No. 21 48 NAE 0007
Guideline(s):	Yes (IOBC (Mead-Briggs *et al.* 2009))
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

ABSTRACT

An extended laboratory study was carried out to determine the effects of the test item GLOB1310aH on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera: Bracconidae). For determination of mortality and reproduction adult wasps were exposed to fresh, dry residues of GLOB1310aH on potted barley plants. Effects on mortality were assessed by the number of surviving, affected, moribund and dead wasps, and effects on reproduction were assessed by the number of parasitised aphids (mummies) produced per female.

The study encompassed 7 treatment groups (5 test item rates, control, reference item), each with 6 replicates. Five females per replicate were exposed to dried residues of GLOB1310aH sprayed on potted barley plants at application rates of 93.75 – 187.5 – 375 – 750 – 1500 mL product/ha with a water volume corresponding to 400 L/ha. Additional test units were treated with deionised water for the water control and with DANADIM PROGRESS (active substance 411.2 g dimethoate/L) as the reference item. Endpoints of the study were the mortality (including determination of the LR_{50}) and additionally effects on reproduction (including determination of the ER_{50}).

In the water-treated control a mortality of 6.7 % was observed. In the test item treatments mortality ranged between 3.3 % and 6.7 %. This resulted in corrected mortality rates between -3.6 % and 0 %. No statistically significant effects on mortality were determined in all test item treatments up to and including 1500 mL product/ha (Chi^2 2x2 Table Test with BONFERRONI Correction, $\alpha = 0.05$). The LR_{50} for GLOB1310aH was estimated to be > 1500 mL product/ha in 400 L water/ha. The NOER (no observed effect rate) for mortality was ≥ 1500 mL product/ha.

The mean number of mummies per female in the test item treatments was between 20.6 and 23.2, and 22.4 mummies per female in the control. No statistically significant effects on reproductive capacity were determined in the test item treatments, up to and included 1500 mL product/ha (WILLIAMS-t-test, α = 0.05). The ER_{50} for GLOB1310aH was estimated to be > 1500 mL product/ha in 400 L water/ha. The NOER (no observed effect rate) for reproduction was ≥ 1500 mL product/ha.

In an extended laboratory study with GLOB1310aH the LR_{50} for *Aphidius rhopalosiphi* was estimated to be > 1500 mL product/ha in 400 L water/ha. The NOER (no observed effect rate) for mortality was ≥ 1500 mL product/ha.
The ER_{50} for GLOB1310aH was estimated to be > 1500 mL product/ha in 400 L water/ha. The NOER (no observed effect rate) for reproduction was ≥ 1500 mL product/ha.

MATERIALS AND METHODS

Test item: GLOB1310aH, batch No.: GLO-20F-2306A
analysed content of a.i.:
Aclonifen: 543.7 g/L (nominal 540 g/L)
Flufenacet: 61.55 g/L (nominal 60 g/L)
Density: 1.2282 g/mL

Test species: Parasitic wasp *Aphidius rhopalosiphi* (DESTEFANI-PEREZ), adults (< 48 hours old)
source (in the stage of mummies): "Katz Biotech AG", An der Birkenpfuhlheide 10, 15837 Baruth, Germany

Test design: Exposure of the adults was achieved via air-dried spray residues on treated, potted barley plants. Seven treatment groups (5 test item rates, water treated control, reference item) were set up with 6 replicates (consisting of 5 females) per treatment.

Mortality assessments were carried out 2, 24 and 48 hours after start of exposure of the wasps. At 48 hours, surviving wasps (15 females per treatment) were removed and their reproductive capacity was assessed by confining them individually over untreated wheat plants infested with adult and nymphal aphids (*Rhopalosiphum padi*). Assessment of reproduction capacity, i.e. number of mummies per female, was made for the control and all treated groups (1 assessment, 14 days after application).

Endpoints: Mortality: number of dead wasps, including the determination of the LR_{50}.
Reproductive capacity: number of mummies per female, including the determination of the ER_{50}.

Reference item: DANADIM PROGRESS
(Dimethoate 411.2 g/L, nominal: 400 g/L)

Test rates: Control (deionised water)
Test item (GLOB1310aH):
93.75 – 187.5 – 375 – 750 – 1500 mL product/ha

The reference item was applied at a rate of 10 mL/ha. All substances were applied in 400 L water/ha. The substances were sprayed on potted barley plants via laboratory spraying equipment and air dried afterwards.

Test conditions:
Temperature: 19-22 °C
relative humidity: 68-72 %
light-dark-cycle: 16 hours light, 8 hours dark
light intensity: 1070 lux (mortality phase)
5360 lx (parasitisation phase)
6720 lx (reproduction phase)
Food: 10 % w/w aqueous fructose solution

Statistics:
Chi^2 2x2 Table Test with BONFERRONI Correction (α = 0.05) for mortality (test item)
Chi^2 2x2 Table Test (α = 0.05) for mortality (reference item)
DUNNETT'S-t-test (α = 0.05) for repellence (test item)
WILLIAMS t-test (α = 0.05) for reproductive capacity (test item)

RESULTS AND DISCUSSION

In the water-treated control a mortality of 6.7 % was observed. In the test item treatments mortality ranged between 3.3 % and 6.7 %. This resulted in corrected mortality rates between -3.6 % and 0 %. No statistically significant effects on mortality were determined in all test item treatments up to and including 1500 mL product/ha (Chi^2 2x2 Table Test with BONFERRONI Correction, α = 0.05). The LR_{50} for GLOB1310aH was estimated to be > 1500 mL product/ha in 400 L water/ha. The NOER (no observed effect rate) for mortality was ≥ 1500 mL product/ha.

The mean number of mummies per female in the test item treatments was between 20.6 and 23.2, and 22.4 mummies per female in the control. No statistically significant effects on reproductive capacity were determined in the test item treatments, up to and included 1500 mL product/ha (WILLIAMS-t-test, α = 0.05). The ER_{50} for GLOB1310aH was estimated to be > 1500 mL product/ha in 400 L water/ha. The NOER (no observed effect rate) for reproduction was ≥ 1500 mL product/ha.

The results are summarised below.

Effects on the parasitic wasp (*Aphidius rhopalosiphi*) exposed to GLOB1310aH in an extended laboratory test

Treatment	**Rate [1] [mL product/ha]**	**Mortality [2] [%]**	**Corrected Mortality [3] [%]**	**Reproduction [4] [mean number of mummies/female]**	**Effects on re-production [5] [%]**
Control	-	6.7	-	22.4	-
Test item	93.75	3.3 (n.s.)	-3.6	21.5 (n.s.)	4.0
Test item	187.5	3.3 (n.s.)	-3.6	20.6 (n.s.)	8.0
Test item	375	6.7 (n.s.)	0	23.2 (n.s.)	-3.6
Test item	750	6.7 (n.s.)	0	22.9 (n.s.)	-2.2
Test item	1500	6.7 (n.s.)	0	22.3 (n.s.)	0.4
	Endpoint [mL product/ha]				
LR_{50}	**> 1500**				
ER_{50}	**> 1500**				

[1] Application rate in 400 L water/ha

[2] Mortality after 48 hours of exposure to the test item on treated barley plants. The results for mortality in individual treatments were compared to that in the control Chi² 2x2 Table Test with BONFERRONI Correction (α = 0.05).
[3] Corrected mortality according to ABBOTT (1925).
[4] Reproduction: mean number of parasitised aphids (mummies)/surviving female. The results were compared to the control by WILLIAMS-t-test (α = 0.05).
[5] Change in mean number of mummies per female, relative to control. A positive value indicates a decrease and a negative value indicates an increase relative to the control.
n.s. not statistically significant different compared to the control

No unusual observations were noted in the control and all test item groups up to and including 1500 mL product/ha at any observation point during the test. There were no statistically significant differences in the behaviour (wasps settled on the plants as a criterion for repellence) in the test item groups up to and including 1500 mL product/ha compared to the control (DUNNETT's-t-test, α = 0.05).

The reference item caused a mortality of 100 % of exposed wasps, resulting in a corrected mortality of 100 %.

CONCLUSION
In an extended laboratory study with GLOB1310aH the LR_{50} for *Aphidius rhopalosiphi* was estimated to be > 1500 mL product/ha in 400 L water/ha. The NOER (no observed effect rate) for mortality was ≥ 1500 mL product/ha.
The ER_{50} for GLOB1310aH was estimated to be > 1500 mL product/ha in 400 L water/ha. The NOER (no observed effect rate) for reproduction was ≥ 1500 mL product/ha.

Comments of zRMS:	The study was conducted to Mead-Briggs *et al.* 2009 guidelines and according to the principles of GLP. No deviations to the guideline were noted. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.2
Report	Effects of GLOB1310aH on the parasitic wasp *Aphidius rhopalosiphi* DESTEFANI-PEREZ in an extended laboratory test (under semi-field conditions aged residues on potted bean plants). Röhlig, U., 2021d. Study No. 21 48 NAR 0001
Guideline(s):	Yes (IOBC (Mead-Briggs *et al.* 2009) modified for an aged residue test)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

ABSTRACT

An extended laboratory study with aged residues on plant surfaces was carried out to determine the effects and the duration of the residual activity of the test item GLOB1310aH on the parasitic wasp *Aphidius rhopalosiphi* (Hymenoptera: Bracconidae). For determination of mortality and reproduction, adult wasps were exposed to fresh dried or under semi-field conditions aged residues of GLOB1310aH on potted bean plants at two exposure times. Effects on mortality were assessed by the

number of surviving, affected, moribund and dead wasps, and effects on reproduction were assessed by the number of parasitised aphids (mummies) produced per female.

The study encompassed one test item rate, one reference item rate and a water treated control, each with 6 replicates for each bioassay. GLOB1310aH was sprayed on potted bean plants (*Phaseolus vulgaris*), variety "Jutta", at an application rate of 2.0 L product/ha with a water volume corresponding to 400 L/ha. Additional test plants were treated with deionised water for the water control and with DANADIM PROGRESS (active substance 411.2 g Dimethoate/L) as the reference item. The application was carried out under semi-field (outdoor) conditions using a spray equipment for small plot applications (plot-sprayer). In the bioassay started on DAT7 detached bean leaf segments were treated in the laboratory with DANADIM PROGRESS with a water volume corresponding to 400 L/ha as the reference item. Endpoints of the study were the mortality and, additionally, effects on reproduction.

The 1st bioassay commenced within 1 hour after treatment of the plants, as soon as the spray residues had dried, i.e. 0 days after treatment (DAT0). A 2nd bioassay was started 7 days after treatment (DAT7). Both bioassays were set up with 6 replicates (consisting of 5 females) per treatment. Exposure of the wasps lasted until 48 hours after start of each bioassay. Mortality assessments were carried out 2, 24 and 48 hours after exposure of the wasps and additionally behavioural impacts were assessed during the initial 3 hours after exposure. In addition, for the control and the test item rates, the reproduction, i.e. number of mummies per female, was determined (1 assessment, 14 days after start of each bioassay).

DAT0:
In the bioassay started on DAT0, in the water-treated control a mortality of 3.3 % was observed. In the test item treatment mortality was 6.7 %. This resulted in a corrected mortality rate of 3.4 %. No statistically significant effects on mortality were determined at the test item rate compared to the control (FISHER`s Exact Binomial test, $\alpha$ = 0.05). In the bioassay initiated on DAT0, the toxic reference item caused a mortality of 100 % of the exposed wasps, resulting in a corrected mortality of 100 % (FISHER`s Exact Binomial test, α = 0.05).

In the bioassay started on DAT0 a reproduction rate of 22.9 mummies/female at the test item rate of 2 L product/ha were obtained. In the control 21.7 mummies/female were observed. Thus an effect on reproduction of -5.5 %, was calculated for the test item treated group compared to the control. No statistically significant effect on reproduction was observed at the 2 L product/ha test item rate (STUDENT-t-test, α = 0.05).

DAT7:
In the bioassay started on DAT7, in the water-treated control a mortality of 6.7 % was observed. In the test item treatment mortality was 3.3 % at 2 L product/ha. This resulted in a corrected mortality rate of -3.6 %. No statistically significant effects on mortality were determined at the test item rate compared to the control (FISHER`s Exact Binomial test, $\alpha$ = 0.05). In the bioassay initiated on DAT7, the toxic reference item caused a mortality of 96.7 % of the exposed wasps, resulting in a corrected mortality of 96.4 % (FISHER`s Exact Binomial test, α = 0.05).

In the bioassay started on DAT7 a reproduction rate of 21.6 mummies/female at the test item rate of 2 L product/ha were obtained. In the control 22.5 mummies/female were observed. Thus an effect on reproduction of 4.0 %, was calculated for the test item treated group compared to the control. No statistically significant effect on reproduction was observed at the 2 L product/ha test item rate (STUDENT-t-test, α = 0.05).

Effects < 50 % on survival and reproduction of *Aphidius rhopalosiphi* were observed in two consecutive bioassays, when the wasps were exposed to fresh dried residues (bioassay started on DAT0) and to 7-day-old residues (bioassay started on DAT7) of GLOB1310aH applied at a rate of 2 L product/ha in 400 L water/ha.

MATERIALS AND METHODS

Test item: GLOB1310aH, batch No.: GLO-20F-2306A

analysed content of a.i.:
Aclonifen: 543.7 g/L (nominal 540 g/L)
Flufenacet: 61.55 g/L (nominal 60 g/L)
density: 1.2282 g/mL

Test species: Parasitic wasp *Aphidius rhopalosiphi* (DESTEFANI-PEREZ), adults (< 48 hours old)
source (in the stage of mummies): "Katz Biotech AG", An der Birkenpfuhlheide 10, 15837 Baruth, Germany,
three days before start of each bioassay.

Test design: Adult wasps were exposed via fresh dried or semi-field aged residues of the test item on potted bean plants. Initial bioassay started on the day of application (DAT0), once residues had dried. The plants intended for the bioassay initiated 7 days after application (DAT7) were maintained outside under rain protection permeable to UV-light. The test comprised 3 treatment groups (1 test item rate, water treated control, reference item).

Treatments were applied to potted bean plants (*Phaseolus vulgaris)* using a spray equipment for small plot applications (plot-sprayer).

Extended laboratory bioassays were initiated within 1 h after treatment application, i.e. 0 days after treatment (DAT0) and 7 days after treatment (DAT7), set up with 6 replicates for test item, control and reference item treatments, consisting of 5 female wasps each. Exposure of the adults was achieved via air-dried residues on treated bean segments. Mortality assessments were carried out 2, 24 and 48 hours after exposure of the wasps. After 48 hours, surviving wasps (15 females per treatment) were removed and their reproductive capacity was assessed by confining them individually over untreated wheat plants infested with adult and nymphal aphids (*Rhopalosiphum padi*). Assessment of reproduction capacity i.e. number of mummies per female was made for the control and test item rates (1 assessment, 14 days after start of each exposure).

Endpoints: Mortality: number of surviving, affected, moribund and dead wasps
Reproductive capacity: number of mummies per surviving female.

Reference item: DANADIM PROGRESS
(411.2 g Dimethoate/L, nominal: 400 g/L)
The reference item was applied at a rate of 30 mL product/ha in 400 L/ha of water on DAT 0 and at a rate of 10 mL product/ha in 400 L/ha of water on DAT7

Test rates: Control (deionised water)
Test item (GLOB1310aH):
2 L product/ha in 400 L/ha of water

Test conditions: <u>Controlled-environment test room:</u>
Temperature: 19 - 22 °C (1st bioassay, DAT0)
19 - 22 °C (2nd bioassay, DAT7)
Relative humidity: 67 - 72 % (1st bioassay, DAT0)
67 - 72 % (2nd bioassay, DAT7)

Light-dark-cycle: 16 hours light, 8 hours dark
Light intensity: <u>(1st bioassay, DAT0)</u>:
1100 lx (exposure phase)

2560 lx (parasitisation phase)
6420 lx (reproduction phase)

Light intensity: (2nd bioassay, DAT7):
1070 lx (exposure phase)
2390 lx (parasitisation phase)
6530 lx (reproduction phase)

Semi-field (outdoor) conditions (non-GLP):
(valid for the full time of ageing)
Temperature (mean/day): 14.5 °C – 20.7 °C
Temperature (min/max): 6.0 °C – 28.2 °C
Relative humidity (mean/day): 56 % - 77 %

Rainfall: 19.9 mm (not relevant, since the treated plants were placed rain-protected under a roof)
Food: 10 % w/w aqueous fructose solution

Statistics: FISHER'S Exact Binomial Test (α = 0.05) for mortality (test item and reference item)
STUDENT-t-test (α = 0.05) for reproductive capacity and for repellence (test item)

RESULTS AND DISCUSSION

DAT0:
In the bioassay started on DAT0, in the water-treated control a mortality of 3.3 % was observed. In the test item treatment mortality was 6.7 %. This resulted in a corrected mortality rate of 3.4 %. No statistically significant effects on mortality were determined at the test item rate compared to the control (FISHER`s Exact Binomial test, $\alpha$ = 0.05). In the bioassay initiated on DAT0, the toxic reference item caused a mortality of 100 % of the exposed wasps, resulting in a corrected mortality of 100 % (FISHER`s Exact Binomial test, α = 0.05).

In the bioassay started on DAT0 a reproduction rate of 22.9 mummies/female at the test item rate of 2 L product/ha were obtained. In the control 21.7 mummies/female were observed. Thus an effect on reproduction of -5.5 %, was calculated for the test item treated group compared to the control. No statistically significant effect on reproduction was observed at the 2 L product/ha test item rate (STUDENT-t-test, α = 0.05).

DAT7:
In the bioassay started on DAT7, in the water-treated control a mortality of 6.7 % was observed. In the test item treatment mortality was 3.3 % at 2 L product/ha. This resulted in a corrected mortality rate of -3.6 %. No statistically significant effects on mortality were determined at the test item rate compared to the control (FISHER`s Exact Binomial test, $\alpha$ = 0.05). In the bioassay initiated on DAT7, the toxic reference item caused a mortality of 96.7 % of the exposed wasps, resulting in a corrected mortality of 96.4 % (FISHER`s Exact Binomial test, α = 0.05).

In the bioassay started on DAT7 a reproduction rate of 21.6 mummies/female at the test item rate of 2 L product/ha were obtained. In the control 22.5 mummies/female were observed. Thus an effect on reproduction of 4.0 %, was calculated for the test item treated group compared to the control. No statistically significant effect on reproduction was observed at the 2 L product/ha test item rate (STUDENT-t-test, α = 0.05).

The results are summarised below.

Effects on the parasitic wasp *Aphidius rhopalosiphi* exposed to fresh dry or under semi-field conditions aged residues of GLOB1310aH in an extended laboratory test

Treatment	Rate [1]	Mortality [2] [%]	Corrected mortality [3] [%]	Reproduction [mean number of mummies/female] [4]	Effect on Reproduction [5] [%]
Bioassay initiated on DAT0 [6]					
Control	-	3.3	-	21.7	-
GLOB1310aH	2 L product/ha	6.7 (n.s.)	3.4	22.9 (n.s.)	-5.5
Reference item DANADIM PROGRESS	30 mL product/ha	100*	100	n.d.	-
Bioassay initiated on DAT7 [6]					
Control	-	6.7	-	22.5	-
GLOB1310aH	2 L product/ha	3.3 (n.s.)	-3.6	21.6 (n.s.)	4.0
Reference item DANADIM PROGRESS	10 mL product/ha	96.7*	96.4	n.d.	-

[1] Application rate in 400 L water/ha.
[2] Mortality after 48 hours of exposure to the test item on treated bean plants. The results for mortality in individual treatments were compared to that in the control using the FISHER'S Exact Binomial Test (α = 0.05) for mortality (test item and reference item)
[3] Corrected mortality according to ABBOTT (1925).
[4] Reproduction: mean number of parasitised aphids (mummies)/surviving female. The results were compared to the control by the STUDENT-t-test (α = 0.05).
[5] Change in mean number of mummies per female, relative to control. A negative value indicates an increase and a positive value indicates a decrease relative to the control.
[6] DAT = Days After Treatment (equivalent to days over which residues aged before bioassay was initiated)
n.s. not statistically significant different compared to the control
* statistically significant different compared to the control
n.d. not determined

In the bioassay started on DAT0 no unusual observations were noted in the control and the test item treated group at any observation point during the test. There were no statistically significant differences in the behaviour (wasps settled on the plants, as a criterion for repellence) in all treated groups in comparison to control (STUDENT-t-test, α = 0.05)

In the bioassay started on DAT7 no unusual observations were noted in the control and the test item treated group at any observation point during the test. There were no statistically significant differences in the behaviour (wasps settled on the plants, as a criterion for repellence) in all treated groups in comparison to control (STUDENT-t-test, α = 0.05).

CONCLUSION

To assess the duration and extent of possible effects of GLOB1310aH on survival and reproduction of the parasitic wasp, *Aphidius rhopalosiphi*, at an application rate of 2 L product/ha, a control (treated with water) and a reference item (DANADIM PROGRESS) were applied to potted bean plants (*Phaseolus vulgaris,* var. "Jutta") under outdoor conditions. After defined time periods, adults of *A. rhopalosiphi* were exposed to the residues on detached bean segments in a series of extended laboratory tests.

Effects < 50 % on survival and reproduction of *Aphidius rhopalosiphi* were observed in two consecutive bioassays, when the wasps were exposed to fresh dried residues (bioassay started on DAT0) and to 7-day-old residues (bioassay started on DAT7) of GLOB1310aH applied at a rate of 2 L product/ha in 400 L water/ha.

Comments of zRMS:	The study was conducted to Blümel *et al.* 2000 guideline and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.2
Report	Effects of GLOB1310aH on the ~~parasitic wasp *Aphidius rhopalosiphi* DESTEFANI PEREZ~~ predatory mite Typhlodromus pyri SCHEUTEN in an extended laboratory test ~~(under semi-field conditions aged residues on potted bean plants).~~ Röhlig, U., 2021f. Study No. 21 48 NTE 0008
Guideline(s):	Yes (IOBC (Blümel *et al.* 2000) modified for the exposure on natural substrate (extended lab test))
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

ABSTRACT

An extended laboratory study was carried out to determine the effects of the test item GLOB1310aH on the predatory mite *Typhlodromus pyri* SCHEUTEN (Acari: Phytoseiidae). For determination of mortality and reproduction, protonymphs of the mites were exposed to fresh, dry residues of GLOB1310aH on bean leaf discs over 14 days. Effects on reproduction were assessed by the number of eggs laid and number of juveniles per evaluation period.

The study encompassed 7 treatment groups (5 test item rates, control, reference item), each with 5 replicates. 20 protonymphs per replicate were exposed to dried residues of GLOB1310aH sprayed on bean leaf discs (*Phaseolus vulgaris*) at application rates of 31.25 – 62.5 – 125 – 250 – 500 mL product/ha with a water volume corresponding to 200 L/ha. Additional test units were treated with deionised water for the water control and with DANADIM PROGRESS (active substance 411.2 g Dimethoate/L) as the reference item. Endpoints of the study were the mortality and additionally effects on reproduction.

After 7 days, in the water-treated control a mortality of 1.0 % was observed. In the test item treatments mortality ranged between 1.0 % and 2.0 %. This resulted in corrected mortality rates between 0 % and 1.0 %. No statistically significant effects on mortality were determined at tested rates, up to including 500 mL product/ha compared to the control (Chi^2-2x2 Table test with BONFERRONI correction, $\alpha = 0.05$). The LR_{50} was estimated to be > 500 mL product/ha. The NOER (no observed effect rate) for mortality was ≥ 500 mL product/ha.

The reproductive capacity of the mites was assessed in the control group and all test item rates. The reproduction rate amounted to 7.43 eggs/female in the control treatment. The reproduction rate in the test item treated groups ranged between 7.17 eggs /female and 7.72 eggs/female. Thus, an effect on reproduction between 3.4 % and -3.9 % was calculated for the test item treated groups compared to the con-

trol. No statistically significant effects on reproduction were determined at all test item rates up to and including 500 mL product/ha (WILLIAMS-t-test, α = 0.05). The ER_{50} was estimated to be > 500 mL product/ha. The NOER (no observed effect rate) for reproduction was ≥ 500 mL product/ha.

In an extended laboratory study with GLOB1310aH the LR_{50} for *Typhlodromus pyri* was estimated to be > 500 mL product/ha in 200 L water/ha. The NOER (no observed effect rate) for mortality was ≥ 500 mL product/ha.
The ER_{50} was estimated to be > 500 mL product/ha. The NOER (no observed effect rate) for reproduction was ≥ 500 mL product/ha.

MATERIALS AND METHODS

Test item:	GLOB1310aH, batch No.: GLO-20F-2306A analysed content of a.i.: Aclonifen: 543.7 g/L (nominal 540 g/L) Flufenacet: 61.55 g/L (nominal 60 g/L) Density: 1.2282 g/mL
Test species:	Predatory mite *Typhlodromus pyri* SCHEUTEN, protonymphs (< 24 hours old); source (in the stage of eggs): "Katz Biotech AG", An der Birkenpfuhlheide 10, 15837 Baruth, Germany
Test design:	Protonymphs were exposed to dried spray residues of different application rates of the test item applied on bean leaf discs (*Phaseolus vulgaris*). 7 treatment groups (5 test item rates, water treated control, reference item) were set up with 5 replicates (consisting of 20 protonymphs) per treatment. Exposure lasted until 14 days after application. Mortality assessments were carried out 3 and 7 days after exposure of the mites and additionally after 9, 11 and 14 days. In addition, for the control and all test item treatment groups of the reproduction, i.e. number of eggs per female, was determined (3 assessments, 9, 11 and 14 days after application).
Endpoints:	Mortality after exposure over 7 days, including determination of a LR_{50} (Lethal Rate 50 %, rate resulting in 50 % mortality) Reproductive capacity of the surviving mites from day 7-14 including determination of an ER_{50} (Effect Rate 50 %, rate resulting in 50 % effect on reproduction)
Reference item:	DANADIM PROGRESS (Dimethoate 411.2 g/L analysed, nominal: 400 g/L)
Test rates:	Control (deionised water) Test item (GLOB1310aH): 31.25 – 62.5 – 125 – 250 – 500 mL product/ha The reference item was applied at a rate of 30 mL/ha (nominally equivalent to 12 g a.s./ha). All substances were applied in 200 L water/ha. The substances were sprayed on bean via laboratory spraying equipment and air dried afterwards.
Test conditions:	Temperature: 23 °C - 27 °C Relative humidity: 68 % - 72 % Light-dark-cycle: 16 hours light : 8 hours dark; Light intensity: 2030 lx Food: pollen: pine (*Pinus nigra*) and birch (*Betula pendula*), 1:1

Statistics: Chi^2-2x2 Table test with BONFERRONI correction (α = 0.05) for mortality (test item)
Chi^2 2x2 Table test (α = 0.05) for mortality (reference item)
WILLIAMS-t-test (α = 0.05) for reproductive capacity

RESULTS AND DISCUSSION

After 7 days, in the water-treated control a mortality of 1.0 % was observed. In the test item treatments mortality ranged between 1.0 % and 2.0 %. This resulted in corrected mortality rates between 0 % and 1.0 %. No statistically significant effects on mortality were determined at tested rates, up to including 500 mL product/ha compared to the control (Chi^2-2x2 Table test with BONFERRONI correction, α = 0.05). The LR_{50} was estimated to be > 500 mL product/ha. The NOER (no observed effect rate) for mortality was ≥ 500 mL product/ha.

The reproductive capacity of the mites was assessed in the control group and all test item rates. The reproduction rate amounted to 7.43 eggs/female in the control treatment. The reproduction rate in the test item treated groups ranged between 7.17 eggs /female and 7.72 eggs/female. Thus, an effect on reproduction between 3.4 % and -3.9 % was calculated for the test item treated groups compared to the control. No statistically significant effects on reproduction were determined at all test item rates up to and including 500 mL product/ha (WILLIAMS-t-test, α = 0.05). The ER_{50} was estimated to be > 500 mL product/ha. The NOER (no observed effect rate) for reproduction was ≥ 500 mL product/ha.

The results are summarised below.

Effects on predatory mite *Typhlodromus pyri* exposed to fresh dry residues of GLOB1310aH in an extended laboratory trial

Treatment	**Rate [1] [mL product/ha]**	**Mortality [2] [%]**	**Corrected mortality [3] [%]**	**Mean number of eggs per female [4] [7-14 Day]**	**Effect on Reproduction [5] [%]**
Control	-	1.0	-	7.43	-
Test item	31.25	1.0 (n.s.)	0	7.72 (n.s.)	-3.9
Test item	62.5	2.0 (n.s.)	1.0	7.17 (n.s.)	3.4
Test item	125	1.0 (n.s.)	0	7.67 (n.s.)	-3.2
Test item	250	1.0 (n.s.)	0	7.53 (n.s.)	-1.3
Test item	500	1.0 (n.s.)	0	7.30 (n.s.)	1.8
	Endpoint [mL product/ha]				
LR_{50}	**> 500**				
NOER	**≥ 500**				
ER_{50}				**> 500**	
NOER				**≥ 500**	

[1] Application rate in 200 L water/ha
[2] Mortality after 7 days of exposure to residues on treated leaf discs. The results for mortality in individual test item treatments were compared to that in the control using the Multiple Sequentially-rejective Chi^2-2x2 Table test after BONFERRONI-HOLM (α = 0.05).
[3] Corrected mortality according to ABBOTT (1925)
[4] Results for reproduction compared by WILLIAMS-t-test (α = 0.05)
[5] Change in mean number of eggs per female, relative to control. A positive value indicates a decrease and a negative value indicates a n increase relative to the control.
n.s. not statistically significant different compared to the control

No unusual observations regarding behaviour were noted in the control and the test item treatment groups at any observation point during the test.

The reference item caused a mortality of 79.0 % of exposed mites, resulting in a corrected mortality of 78.8 %.

CONCLUSION

In an extended laboratory study with GLOB1310aH the LR_{50} for *Typhlodromus pyri* was estimated to be > 500 mL product/ha in 200 L water/ha. The NOER (no observed effect rate) for mortality was ≥ 500 mL product/ha.
The ER_{50} was estimated to be > 500 mL product/ha. The NOER (no observed effect rate) for reproduction was ≥ 500 mL product/ha.

Comments of zRMS:	The study was conducted to Blümel *et al.* 2000 guideline and according to the principles of GLP. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.3.2
Report	Effects of GLOB1310aH on the ~~parasitic wasp *Aphidius rhopalosiphi* DESTEFANI-PEREZ~~ predatory mite Typhlodromus pyri SCHEUTEN in an extended laboratory test (under semi-field conditions aged residues on potted bean plants). Röhlig, U., 2021g. Study No. 21 48 NTR 0001
Guideline(s):	Yes (IOBC (Blümel *et al.* 2000) ~~modified for the exposure on natural substrate (extended lab test))~~ modified for extended aged-residue conditions
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

- **ABSTRACT**

An extended laboratory study with aged residues on plant surfaces was carried out to determine the effects and the duration of the residual activity of the test item GLOB1310aH on the predatory mite *Typhlodromus pyri* SCHEUTEN (Acari: Phytoseiidae). For determination of mortality and reproduction, protonymphs of the predatory mites were exposed in the laboratory to fresh dried or under semi-field conditions aged residues of GLOB1310aH, on detached bean leaves at three exposure times. Effects on mortality were assessed by the number of surviving, dead and escaped predatory mites. Effects on reproduction were assessed by the number of eggs laid per female and number of juveniles per evaluation period.

The study encompassed 3 treatment groups (1 test item rate, control, reference item), each with 5 replicates. 20 protonymphs per replicate were exposed to fresh dried or under semi-field conditions aged residues of the test item sprayed on potted bean plants (*Phaseolus vulgaris*), variety “Jutta”, at an application rate of 2 L product/ha with a water volume corresponding to 400 L/ha. Additional test plants were treated with deionised water for the water control in the same way as the test item groups. A reference item group with DANADIM PROGRESS (active substance 411.2 g Dimethoate/L) was treated on DAT0 under semi-field (outdoor) conditions. On DAT7 and on DAT14, the reference item was freshly applied on excised untreated bean leaves under laboratory conditions. Endpoints of the study were the mortality and additionally effects on reproduction.

Exposure lasted until 14 days after the start of each bioassay. Mortality assessments were carried out 3 and 7 days after exposure of the mites and, additionally, after 7, 9, 11 and 14 days, the number of males and females were counted. In addition, the reproduction, *i.e.* number of eggs per female, was determined (3 assessments on 9, 11 and 14 days after start of bioassay) for the control and the test item treatment.

DAT0:
In the bioassay started on DAT0, in the water-treated control a mortality of 6.0 % was observed after 7 days. In the test item treatment, mortality was 93.0 % at 2 L product/ha. This resulted in a corrected mortality rate of 92.6 %. Statistically significant effects on mortality were determined at the test item rate of 2 L product/ha compared to the control (Chi^2 2x2 Table test, α = 0.05). In the bioassay initiated on DAT0, the toxic reference item caused a mortality of 100 % of the exposed mites after 7 days, resulting in a statistically significant corrected mortality of 100 % (Chi^2 2x2 Table test, α = 0.05).

In the bioassay started on DAT0 no reproduction test was started, as > 50 % corrected mortality occurred in the test item treatment group.

DAT7:
In the bioassay started on DAT7, in the water-treated control a mortality of 2.0 % was observed after 7 days. In the test item treatment, mortality was 2.0 % at 2 L product/ha. This resulted in a corrected mortality rate of 0 %. No statistically significant effects on mortality were determined at the test item rate of 2 L product/ha compared to the control (Chi^2 2x2 Table test, α = 0.05). In the bioassay initiated on DAT7, the toxic reference item caused a mortality of 82.0 % of the exposed mites after 7 days, resulting in a statistically significant corrected mortality of 81.6 % (Chi^2 2x2 Table test, α = 0.05).
In the bioassay started on DAT7, the reproduction rate in the 2 L product/ha test item treated group resulted in 6.40 eggs/female, compared to 6.39 eggs/female in the control. Thus, an effect on reproduction of -0.2 % was calculated for the test item treated group compared to the control. No statistically significant effects on reproduction were observed at the 2 L product/ha test item treatment group (STUDENT-t-test, α = 0.05).

DAT14:
In the bioassay started on DAT14, in the water-treated control a mortality of 3.0 % was observed after 7 days. In the test item treatment, mortality was 2.0 % at 2 L product/ha. This resulted in a corrected mortality rate of -1.0 %. No statistically significant effects on mortality were determined at the test item rate of 2 L product/ha compared to the control (Chi^2 2x2 Table test, α = 0.05). In the bioassay initiated on DAT14, the toxic reference item caused a mortality of 79.0 % of the exposed mites after 7 days, resulting in a statistically significant corrected mortality of 78.4 % (Chi^2 2x2 Table test, α = 0.05).
In the bioassay started on DAT14, the reproduction rate in the 2 L product/ha test item treated group resulted in 6.70 eggs/female, compared to 6.74 eggs/female in the control. Thus, an effect on reproduction of 0.6 % was calculated for the test item treated group compared to the control. No statistically significant effects on reproduction were observed at the 2 L product/ha test item treatment group (STUDENT-t-test, α = 0.05).

Under extended laboratory conditions, the effects on mortality and reproduction of the predatory mite *Typhlodromus pyri* exposed to aged spray residues on detached bean leaf discs at a rate of 2 L GLOB1310aH/ha were below 50 % at 7 and 14 days after treatment (DAT7 and DAT14).

MATERIALS AND METHODS

Test item:	GLOB1310aH, batch No.: GLO-20F-2306A analysed content of a.i.: Aclonifen: 543.7 g/L (nominal 540 g/L) Flufenacet: 61.55 g/L (nominal 60 g/L) density: 1.2282 g/mL

Test species: Predatory mite *Typhlodromus pyri* SCHEUTEN (protonymphs < 24 hours old) source: Katz Biotech AG, An der Birkenpfuhlheide 10, 15837 Baruth, Germany

Test design: Protonymphs were exposed via freshly dried or aged residues of the test item on bean leaves. The test comprised 3 treatment groups on DAT0, DAT7 and DAT14 (1 test item rate, water treated control, reference item) set up with 5 replicates (consisting of 20 protonymphs per replicate).

Treatments were applied to potted bean plants using a spray equipment for commercial applications (plot-sprayer). For each bioassay, the replicate leaves were gently cut to leaf discs, which were placed with the treated side upward on moistened cotton wool in Petri dishes.

The ageing of spray residues on potted bean plants took place under semi-field (outdoor) conditions with rain protection (under a UV-permeable roof) from the application until the start of the respective bioassay.

Exposure lasted until 14 days after start of each bioassay. Mortality assessments were carried out 3 and 7 days after exposure of the mites and additionally after 7, 9, 11 and 14 days after the number of females and males was counted. In addition, the reproduction, *i.e.* number of eggs per female, was determined (3 assessments on 9, 11 and 14 days after start of bioassay).

Extended laboratory bioassays were initiated 0, 7 and 14 days after the application (DAT0, DAT7 and DAT14)

Endpoints: Mortality: number of surviving, dead, escaped mites (trapped or not found) and after start of each bioassay over 7 days
Reproduction: number of eggs laid and number of juveniles per evaluation period per female from day 7-14

Test rates: Control (deionised water): 400 L/ha
Test item (GLOB1310aH):
2 L product/ha in 400 L/ha of deionised water
Reference item (DANADIM PROGRESS, 411.2 g Dimethoate/L):
200 mL product/ha (nominally equivalent to 80 g a.i./ha) in 400 L/ha of deionised water, DAT0)
30 mL product/ha (nominally equivalent to 12 g a.i./ha) in 200 L/ha of deionised water, DAT7 and DAT14)

Test conditions:

<u>Controlled-environment test room:</u>

Temperature:	23 °C – 27 °C
Relative humidity:	66 % – 72 %
Light-dark-cycle:	16 hours light, 8 hours dark
Light intensity:	1980 – 2050 lx
Food:	pollen: pine *(Pinus nigra)* and birch *(Betula pendula)* 1:1

<u>Outdoor weather conditions (non-GLP):</u>
(valid for the full time of ageing)

Temperature (mean/day):	10.3 °C – 17.1 °C
Temperature (min/max):	5.8 °C – 23.0 °C
Relative humidity (mean/day):	56 % - 90 %

	Rainfall:	68.5 mm (not relevant, since the treated plants were placed rain-protected under a roof)
Statistics:	Chi^2 2x2 Table test (α = 0.05) for mortality (test item) Chi^2 2x2 Table test (α = 0.05) for mortality (reference item) Student-t-test (α = 0.05) for reproduction (DAT7, DAT14) ToxRat Professional 3.3.0 (RATTE 2018)	

RESULTS AND DISCUSSION

DAT0:
In the bioassay started on DAT0, in the water-treated control a mortality of 6.0 % was observed after 7 days. In the test item treatment, mortality was 93.0 % at 2 L product/ha. This resulted in a corrected mortality rate of 92.6 %. Statistically significant effects on mortality were determined at the test item rate of 2 L product/ha compared to the control (Chi^2 2x2 Table test, α = 0.05). In the bioassay initiated on DAT0, the toxic reference item caused a mortality of 100 % of the exposed mites after 7 days, resulting in a statistically significant corrected mortality of 100 % (Chi^2 2x2 Table test, α = 0.05).

In the bioassay started on DAT0 no reproduction test was started, as > 50 % corrected mortality occurred in the test item treatment group.

DAT7:
In the bioassay started on DAT7, in the water-treated control a mortality of 2.0 % was observed after 7 days. In the test item treatment, mortality was 2.0 % at 2 L product/ha. This resulted in a corrected mortality rate of 0 %. No statistically significant effects on mortality were determined at the test item rate of 2 L product/ha compared to the control (Chi^2 2x2 Table test, α = 0.05). In the bioassay initiated on DAT7, the toxic reference item caused a mortality of 82.0 % of the exposed mites after 7 days, resulting in a statistically significant corrected mortality of 81.6 % (Chi^2 2x2 Table test, α = 0.05).
In the bioassay started on DAT7, the reproduction rate in the 2 L product/ha test item treated group resulted in 6.40 eggs/female, compared to 6.39 eggs/female in the control. Thus, an effect on reproduction of -0.2 % was calculated for the test item treated group compared to the control. No statistically significant effects on reproduction were observed at the 2 L product/ha test item treatment group (STUDENT-t-test, α = 0.05).

DAT14:
In the bioassay started on DAT14, in the water-treated control a mortality of 3.0 % was observed after 7 days. In the test item treatment, mortality was 2.0 % at 2 L product/ha. This resulted in a corrected mortality rate of -1.0 %. No statistically significant effects on mortality were determined at the test item rate of 2 L product/ha compared to the control (Chi^2 2x2 Table test, α = 0.05). In the bioassay initiated on DAT14, the toxic reference item caused a mortality of 79.0 % of the exposed mites after 7 days, resulting in a statistically significant corrected mortality of 78.4 % (Chi^2 2x2 Table test, α = 0.05).
In the bioassay started on DAT14, the reproduction rate in the 2 L product/ha test item treated group resulted in 6.70 eggs/female, compared to 6.74 eggs/female in the control. Thus, an effect on reproduction of 0.6 % was calculated for the test item treated group compared to the control. No statistically significant effects on reproduction were observed at the 2 L product/ha test item treatment group (STUDENT-t-test, α = 0.05).

The results are summarised below.

Effects on the predatory mite (*Typhlodromus pyri*) exposed to fresh and aged residues of GLOB1310aH in an extended laboratory trial

Treatment	Rate [1]	Mortality [2] [%]	Corrected mortality [3] [%]	Reproduction [4] [mean number of eggs/female]	Effects on Reproduction [5] [%]
Bioassay initiated DAT0 [6]					
Control	-	6.0	-	6.44	-
Test item	2 L product/ha	93.0*	92.6	n.d.	-
Reference item	200 mL product/ha	100*	100	-	-
Bioassay initiated DAT7 [6]					
Control	-	2.0	-	6.39	-
Test item	2 L product/ha	2.0 (n.s.)	0	6.40 (n.s.)	-0.2
Reference item	30 mL product/ha	82.0*	81.6	-	-
Bioassay initiated DAT14 [6]					
Control	-	3.0	-	6.74	-
Test item	2 L product/ha	2.0 (n.s.)	-1.0	6.70 (n.s.)	0.6
Reference item	30 mL product/ha	79.0*	78.4	-	-

Test item: GLOB1310aH

[1] Application rate in 400 L water/ha (control, test item, reference item DAT0)
Application rate in 200 L water/ha (reference item, DAT7 and DAT14)
[2] Mortality: percentage of individuals (after 7 days of each exposure)
[3] Corrected mortality according to ABBOTT (1925)
[4] Reproduction: mean number of eggs per female.
[5] Change in mean numbers of eggs per female, relative to control. A positive value indicates a decrease and a negative value indicates an increase relative to the control.
[6] DAT = Days After Treatment (equivalent to days over which residues aged before bioassay was initiated)
n.d. not determined (corrected mortality > 50 %)
n.s. not statistically significantly different compared to the corresponding control
* statistically significantly different compared to the corresponding control

No unusual observations regarding behavior were noted in the control and the test item treatment groups at any observation point during the test.

CONCLUSION

The duration and the extent of effects of aged residues of GLOB1310aH applied on potted bean plants (*Phaseolus vulgaris,* var. “Jutta”) on the predatory mite *Typhlodromus pyri* were evaluated under extended laboratory test conditions.

Under extended laboratory conditions, the effects on mortality and reproduction of the predatory mite *Typhlodromus pyri* exposed to aged spray residues on detached bean leaf discs at a rate of 2 L GLOB1310aH/ha were below 50 % at 7 and 14 days after treatment (DAT7 and DAT14).

A 2.4 KCP 10.4 Effects on non-target soil meso- and macrofauna

A 2.4.1 KCP 10.4.1 Earthworms

Comments of zRMS:	The study was performed according to OECD TG 222 and principles of GLP. The validity criteria are met. For the control group: - Adult mortality: ≤ 10 % (being 0.0 % after 4 weeks)

	- Number of juveniles per replicate: ≥ 30 (being 252 to 344) - Coefficient of variation of reproduction: ≤ 30 % (being 10.6 %). The study is considered acceptable and suitable for the risk assessment.

Reference:	KCP 10.4.1
Report	Effects of GLOB1310aH on the reproduction of the earthworm *Eisenia fetida* in artificial soil. Friedrich S., 2021a. Study No. 21 48 TEC 0003
Guideline(s):	Yes (OECD 222 (2016))
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

SUMMARY

Study title:	Effects of GLOB1310aH on the reproduction of the earthworm *Eisenia fetida* in artificial soil
Guideline:	OECD 222 (2016)
Name of the test item:	GLOB1310aH
Batch No.:	GLO-20F-2306A
Active ingredient/ content:	aclonifen 540 g/L (nominal), 543.7 g/L (analysed) flufenacet 60 g/L (nominal), 61.55 g/L (analysed)
Test species:	earthworm *Eisenia fetida* (Savigny, 1826)
Test design:	Effects on earthworms: 56 days; 8 test item treatment groups and an untreated control group, 8 replicates in the control group and 4 replicates in the test item treatment, 10 worms per replicate; assessment of adult worm mortality, behavioural effects and biomass development after 28 days, reproduction rate after an additional 28 days (assessed 56 days after application)
Test system:	Exposure of worms to different concentrations of the test item mixed into artificial soil substrate (with 10 % peat)
Reference item:	Maypon Flow (Carbendazim, SC 500) The effects of the reference item were investigated in a separate study.
Test conditions:	Temperature: 18.0 - 21.1 °C Light intensity: 590 lux Photoperiod: light : dark = 16 h : 8 h
Treatments:	Control (untreated), test item (GLOB1310aH)
Test concentrations:	3.33, 6.0, 10.8, 19.4, 35.0, 63.0, 113.4, 204.1 mg test item/kg soil dry weight (spacing factor: 1.8)
Dates of work:	Experimental start date: 17 December 2020

Experimental completion date: 11 February 2021

Statistics: Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, (α = 0.05, one-sided greater), Williams-t-test for biomass change and reproduction (α = 0.05, one-sided smaller),
Logit analysis for calculation of for calculation of EC_x;
Statistical program: ToxRat Professional 3.3.0 (2018)

Results:

The test item caused no statistically significant effect (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater) on mortality and no statistically significant change in biomass (change in fresh weight after 4 weeks relative to initial fresh weight) compared to the control group at any concentration tested (Williams-t-test, α = 0.05, one-sided smaller). Statistically significant effects (Williams-t-test, α = 0.05, one-sided smaller) on the number of juveniles compared to the control group were recorded at concentrations of 113.4 and 204.1 mg test item/kg soil d.w.

Effects of GLOB1310aH on *Eisenia fetida* in a 56-day reproduction study

Endpoint	Treatment group (mg test item/kg soil d.w.)								
	Control	3.33	6.0	10.8	19.4	35.0	63.0	113.4	204.1
Mortality of adult worms after 4 weeks (%)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5
Mean biomass change after 4 weeks (%)	26.6	25.8	27.1	24.8	29.4	26.0	28.9	27.3	26.3
Mean number of juveniles after 8 weeks	300.0	303.3	289.3	308.8	305.5	291.5	298.8	254.5*	203.8*
Reduction of reproduction compared to control (%)	-	-1.1	3.6	-2.9	-1.8	2.8	0.4	15.2	32.1
	Endpoint (mg test item/kg soil d.w.)								
NOEC (mortality)	204.1								
NOEC (biomass)	204.1								
NOEC (reproduction)	63.0								
LC_{50} (mortality) [1]	> 204.1								
EC_{10} (reproduction) [2]	98.5 (95 % confidence limits 76.7 – 126.6)								
EC_{20} (reproduction) [2]	147.2 (95 % confidence limits 128.6 – 168.5)								
EC_{50} (reproduction) [2]	> 204.1								

Not statistically significantly different to control regarding mortality (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater) and biomass (Williams-t-test, α = 0.05, one-sided smaller)
* statistically significantly different compared to control regarding reproduction (Williams-t-test, α = 0.05, one-sided smaller)
Negative values = increase, relative to control
[1] based on estimation of the data,
[2] Logit analysis

criteria for the control group were met:
- Adult mortality: ≤ 10 % (being 0.0 % after 4 weeks)
- Number of juveniles per replicate: ≥ 30 (being 252 to 344)
- Coefficient of variation of reproduction: ≤ 30 % (being 10.6 %)

CONCLUSION
In a 56-day earthworm reproduction study with GLOB1310aH, no statistically significant effect on survival of the adult earthworms and no statistically significant effects on biomass of the earthworm *Eisenia fetida* in artificial soil were determined up to and including 204.1 mg test item/kg soil dry weight weight, i.e. the highest concentration tested.
The NOEC for mortality and change of biomass was determined to be 204.1 mg test item/kg soil dry

weight. The NOEC for reproduction was determined to be 63.0 mg test item/kg soil dry weight. The EC_{10}, EC_{20} and EC_{50} values for reproduction were calculated to be 98.5, 147.2 and > 204.1 mg test item/kg soil dry weight.

A 2.4.2 KCP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

Comments of zRMS:	The study was performed according to OECD TG 232 and principles of GLP. The validity criteria are met. For the control group: · Mean adult mortality: ≤ 20 % (observed: 3.8 %) · Mean number of juveniles per test vessel: ≥100 (observed: average of 1130/vessel) · Coefficient of variation for the mean number of juveniles: < 30 % (observed: 9.7 %) The study is considered acceptable and suitable for the risk assessment.

Reference:	KCP 10.4.2
Report	Effects of GLOB1310aH on the reproduction of the collembolan *Folsomia candida*. Friedrich S., 2021b. Study No. 21 48 TCC 0002
Guideline(s):	Yes (OECD TG 232 (2016))
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

Materials & methods

Test item:	GLOB1310aH	
Batch No.:	GLO-20F-2306A	
Active ingredient/ content:	aclonifen	540 g/L (nominal), 543.7 g/L (analysed)
	flufenacet	60 g/L (nominal), 61.55 g/L (analysed)
Test species:	Collembola (*Folsomia candida*), age: 9 - 12 days; source: in-house culture.	
Test design:	Effects on *Folsomia candida*: 28 days; 8 test item treatment groups and an untreated control group, 8 replicates in the control group and 4 replicates in the test item treatment groups, each containing 10 collembolans; assessments of adult mortality and reproduction 28 days after application	
Endpoints:	Mortality and reproduction after 28 days	
Test system:	Exposure of collembolans to different concentrations of the test item mixed into the substrate (artificial soil with 5 % peat)	
Reference item:	Boric acid The effects of the reference item were investigated in a separate study.	

Test conditions:	Temperature:	18.0 - 20.0 °C
	Light intensity:	590 lux
	Photoperiod:	light : dark = 16 h : 8 h
Treatments:	Control (untreated), test item (GLOB1310aH)	
Test concentrations:	15.6, 23.3, 35.0, 52.5, 78.8, 118.1, 177.2, 265.8 mg test item/kg soil dry weight (spacing factor: 1.5)	
Dates of work:	Experimental start date:	06 January 2021
	Experimental completion date:	03 February 2021
Statistics:	Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, Williams-t-test (α = 0.05, one-sided), Statistical program: ToxRat Professional 3.3.0 (2018)	

Results & discussion

No statistically significant effects on parental mortality (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater) and on the number of juveniles (Williams-t-test, α = 0.05, one-sided smaller) compared to the control group were found at any concentration tested.

Results are summarised in Table 1.

Table 16: Chronic effects of GLOB1310aH on *Folsomia candida* in a 28-day reproduction study

Endpoint	**Treatment group [mg test item/kg soil dry weight]**								
	Control	15.6	23.3	35.0	52.5	78.8	118.1	177.2	265.8
Mean adult mortality [%]	3.8	2.5	2.5	0.0	2.5	0.0	0.0	5.0	7.5
Mean number of juveniles	1130	1134	1130	1141	1121	1165	1173	1099	1022
Reduction of reproduction [%] compared to control	-	-0.4	0.0	-1.0	0.8	-3.1	-3.8	2.8	9.6
	Endpoints [mg test item/kg soil dry weight]								
NOEC (mortality)	265.8								
NOEC (reproduction)	265.8								
LC_{50} (mortality) [1]	> 265.8								
EC_{10} (reproduction) [1]	> 265.8								
EC_{20} (reproduction) [1]	> 265.8								
EC_{50} (reproduction) [1]	> 265.8								

Not statistically significantly different to control regarding mortality (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater) and reproduction (Williams-t-test, α = 0.05, one-sided smaller)
Calculations were done using unrounded values
Negative values = increase, relative to control
[1] based on estimation of the data

In a separate study (BioChem project No. 20 48 TCC 0064, dated 14 September 2020), the EC_{50} (reproduction) of the reference item boric acid was calculated to be 107 mg/kg soil dry weight. The results of the reference test demonstrate the sensitivity of the test system

The validity criteria for the control group were met:
- Mean adult mortality: ≤ 20 % (observed: 3.8 %)
- Mean number of juveniles per test vessel: ≥100 (observed: average of 1130/vessel)
- Coefficient of variation for the mean number of juveniles: < 30 % (observed: 9.7 %)

Conclusion

In a 28-day *Folsomia candida* reproduction study with GLOB1310aH, the NOEC for mortality of the parental collembolans was determined to be 265.8 mg test item/kg soil dry weight. The LC_{50} could not be calculated, but it can be concluded that the LC_{50} is higher than 265.8 mg test item/kg soil d.w., the highest concentration tested.
The NOEC for reproduction was determined to be 265.8 mg test item/kg soil dry weight. The EC_{10}, EC_{20} and EC_{50} values for reproduction could not be calculated, but it can be concluded that these values are higher than 265.8 mg test item/kg soil d.w., the highest concentration tested.

Comments of zRMS:	The study was performed according to OECD TG 226 and principles of GLP. The validity criteria are met. For the control group: - Mean mortality of adult females: ≤ 20 % (observed: 0.0 %) - Mean number of juveniles per replicate: ≥ 50 (observed: 306.4) - Coefficient of variation (mean number of juveniles per replicate): ≤ 30 % (observed: 6.0 %) The study is considered acceptable and suitable for the risk assessment.

Reference:	KCP 10.4.2
Report	Effects of GLOB1310aH on the reproduction of the predatory mite *Hypoaspis aculeifer*. Schulz, L., 2021a. Study No. 21 48 THC 0001
Guideline(s):	Yes (OECD TG 226 (2016))
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

SUMMARY

Study title:	Effects of GLOB1310aH on the reproduction of the predatory mite *Hypoaspis aculeifer*	
Guideline:	OECD 226 (2016)	
Name of the test item:	GLOB1310aH	
Batch No.:	GLO-20F-2306A	
Active ingredients/content:	aclonifen flufenacet	540 g/L (nominal), 543.7 g/L (analysed) 60 g/L (nominal), 61.55 g/L (analysed)
Test species:	*Hypoaspis aculeifer* (CANESTRINI) age: adult female mites with an age difference of 3 days source: Katz Biotech AG, Baruth	

Test system:	Exposure of female mites to different concentrations of the test item mixed into artificial soil substrate
Test design:	The effects of the test item on mortality and reproduction of the soil mite species *Hypoaspis aculeifer* (CANESTRINI) were investigated in a chronic laboratory experiment over a time period of 14 days according to OECD 226. Each of the eight different test item concentrations were homogeneously mixed into artificial soil and filled into glass vessels. Subsequently, the soil mites were introduced on top of the soil and the vessels were covered. Four replicates were performed for the test item groups and eight replicates for the control group; each replicate consisted of ten female soil mites. The mites were fed with *Tyrophagus putrescentiae* (SCHRANK) at the beginning and every two to three days during the whole test period. For the main measured variables, the number of juveniles per test vessel and additionally the mortality of the adult female mites were determined. Mortality and reproductive output of the mites exposed to the test item were compared to that of the control in order to determine the no observed effect concentration (NOEC). Assessment of adult mortality and reproduction effects was carried out after 14 days.
Endpoints:	Mortality of adults and number of juveniles
Reference item:	Dimethoate 400 EC (400 g/L, nominal). Test concentrations: 0.9, 1.3, 2.0, 3.0, 4.4, 6.7, 10.0, 15.0 mg a.s./kg soil dry weight (d.w.) nominally equivalent to 2.3, 3.5, 5.3, 7.9, 11.9, 17.8, 26.7, 40.1 mg reference item/kg soil d.w. (spacing factor 1.5) The effects of the reference item were investigated in a separate study.
Test concentrations:	16.7, 30.0, 54.0, 97.2, 175.0, 314.9, 566.9 ,1020.4 mg test item/kg soil dry weight (spacing factor: 1.8)
Test conditions:	Artificial soil according to OECD 226, pH 6.4 - 6.5 at test start, pH 6.1 - 6.3 at test end; water content at test start 46.98 - 48.81 % of maximum water holding capacity (WHC) and 48.31 - 50.23 % of maximum WHC at test end; temperature 19.5 - 21.4 °C; photoperiod: 16 h light : 8 h dark; light intensity: 523 lux.
Dates of work:	Experimental start date: 25.01.2021 Experimental completion date: 18.02.2021
Statistics:	<u>Mortality</u> Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm (α = 0.05, one-sided greater) <u>Reproduction</u> Multiple Sequentially-rejective Welch-t-test After Bonferroni-Holm (α = 0.05, one-sided smaller), Logit analysis using linear max. likelihood regression Statistical program: ToxRat Professional 3.3.0 (RATTE 2018)

RESULTS

All validity criteria for the study were met.

Mortality rates of 0.0 - 7.5 % were recorded in the test item treatment groups. In the control group, the mortality rate was 0.0 %.
The observed mortality rates in the test item treatment groups compared to control were not statistically significant (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm, α = 0.05, one-sided greater). Differences in the behaviour and the morphology of the mites between the control and the test item treatment groups could not be observed.

Fourteen days after introduction of the parental mites into the test vessels, the mean number of juveniles was 307.8, 295.5, 285.5, 310.3, 297.0, 281.8, 268.8 and 262.0 at concentrations 16.7, 30.0, 54.0, 97.2, 175.0, 314.9, 566.9 and 1020.4 mg test item/kg soil d.w., respectively. The mean reproduction in the control reached 306.4 juveniles. The test item showed no statistically significantly adverse effects on reproduction up to and including 566.9 mg test item/kg soil d.w. However, the test item caused statistically significant effects on reproduction at 1020.4 mg test item/kg soil d.w. (Multiple Sequentially-rejective Welch-t-test After Bonferroni-Holm, α = 0.05, one-sided smaller).

In a separate study (BioChem project No. 20 48 THC 0054, experimental start date: 19.08.2020, reported 10.11.2020), the EC_{50} (reproduction) of the reference item Dimethoate 400 EC (400 g/L nominal) was calculated to be 4.71 mg a.s./kg soil d.w. The results of the reference test demonstrate the sensitivity of the test system.

Table 17: Effects of the test item on *Hypoaspis aculeifer* mortality and reproduction (day 14)

Endpoint	**Treatment group [mg test item/kg soil dry weight]**								
	Control	**16.7**	**30.0**	**54.0**	**97.2**	**175.0**	**314.9**	**566.9**	**1020.4**
Mean adult mortality [%] (day 14)	0.0	2.5	0.0	0.0	2.5	0.0	2.5	2.5	7.5
Mean number of juveniles (day 14)	306.4	307.8	295.5	285.5	310.3	297.0	281.8	268.8	262.0*
Coefficient of variation [%]	6.0	2.8	8.5	2.6	4.0	2.3	14.6	8.3	4.9
Reproduction in [%] of control	100	100	96	93	101	97	92	88	86
	Endpoint [mg test item/kg soil dry weight]								
NOEC (mortality)	≥ 1020.4								
NOEC (reproduction)	566.9								
LC_{50} (mortality) [1]	> 1020.4								
EC_{10} (reproduction) [2]	502.0 (95 % confidence limit 292.4 - 862.0)								
EC_{20} (reproduction) [1]	> 1020.4								
EC_{50} (reproduction) [1]	> 1020.4								

Not statistically significantly different compared to the control (Multiple Sequentially-rejective Fisher Test after Bonferroni-Holm for mortality, α = 0.05, one-sided greater)
* statistically significantly different compared to the control (Multiple Sequentially-rejective Welch-t-test After Bonferroni-Holm, α = 0.05, one-sided smaller)
[1] based on estimation of the data
[2] based on Logit analysis using max. likelihood regression

CONCLUSION

In a 14-day *Hypoaspis aculeifer* reproduction study with GLOB1310aH, the LC_{50} for mortality could not be calculated, but it can be concluded, that this value is higher than 1020.4 mg test item/kg soil dry weight. The EC_{10} value for reproduction was calculated to be 502.0 mg test item/kg soil dry weight. The NOEC for mortality and reproduction was determined to be ≥ 1020.4 and 566.9 mg test item/kg soil d.w., respectively.

A 2.4.2.1 KCP 10.4.2.1 Species level testing

A 2.4.2.2 KCP 10.4.2.2 Higher tier testing

A 2.5 KCP 10.5 Effects on soil nitrogen transformation

Comments of zRMS:	The study was conducted to OECD guideline 216 and according to the principles of GLP. All the validity criterion are met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.5
Report	Effects of GLOB1310aH on the activity of soil microflora (Nitrogen transformation test). Schulz, L., 2021b. Study No. 21 48 SMN 0001
Guideline(s):	Yes (OECD TG 216 (2000))
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

SUMMARY

Test item:	GLOB1310aH
Study title:	Effects of GLOB1310aH on the activity of soil microflora (Nitrogen transformation test)
Guideline(s):	OECD 216 (2000)
Name of the test item:	GLOB1310aH
Batch No.:	GLO-20F-2306A
Formulation type:	SC
Active ingredient/content:	aclonifen 540 g/L (nominal); 543.7g/kg (analysed) flufenacet 60 g/L (nominal); 61.55 g/L (analysed)
Test soil:	Biologically active agricultural soil: loamy sand (DIN 4220) / loam (USDA), pH 6.1, 1.42 % C_{org}, WHC: 38.20 g/100 g dry soil.
Test design: (2000).	The test was performed in accordance with the OECD Guideline 216 Aim of the study was the determination of the nitrogen transformation (NO_3-nitrogen-production) in soil enriched with lucerne meal (concentration in soil 0.5 %) by comparison of nitrogen transformation in test item treated soil with a non-treated soil.

and cal).	Three replicates per treatment and concentration. NH_4-nitrogen, NO_3-NO_2-nitrogen were determined by using the Autoanalyzer (SEAL Analyti-
	Sampling scheme: 0, 7, 14 and 28 days after treatment.
Test concentrations:	Control, 3.8 mg test item/kg soil dry weight and 19 mg test item/kg soil dry weight. Test concentrations related to a soil depth of 5 cm and a soil density of 1.5 g/cm^3.
Endpoints:	Effects on NO_3-nitrogen-production after 28 days of exposure.
Reference item: in a	Dinoterb (purity: 99.28 % (g/g) analysed). The reference item was tested separate study (20 48 SMO 0001) at concentrations of 6.80, 13.60 and 27.20 mg/kg soil dry weight.
Test conditions: ty;	Water content: approximately 45 % of its maximum water holding capaci-
	water content: 16.40 - 16.92 g/100 g dry soil; pH: 6.0 Soil samples were incubated at 18.9 - 20.5 °C, while stored in
test vessels in	
	the dark.
Statistics: cients	Calculation of mean values per treatment, standard deviations, coeffi- of variation.
Dates of work:	Experimental start: 23.11.2020 Experimental end: 21.12.2020

RESULTS

No adverse effects of the test item on nitrogen transformation in soil could be observed at both test concentrations (3.8 mg/kg soil dry weight and 19 mg/kg soil dry weight) after 28 days (time interval 14-28). The results are summarised in the table below.

Table 18: Effects on nitrogen transformation in soil after treatment with the test item

Time Interval (days)	Control	3.8 mg GLOB1310aH/ kg soil dry weight		19 mg GLOB1310aH/ kg soil dry weight	
	NO_3-N/day [mg/kg soil d.w.]	NO_3-N/day [mg/kg soil d.w.]	% difference to control [1)]	NO_3-N/day [mg/kg soil d.w.]	% difference to control [1)]
0-7	3.23	3.19	-1.5	3.75	+16.1
7-14	1.61	1.46	-9.7	0.90	-44.0
14-28	1.43	1.49	+4.0	1.51	+5.7

The calculations were performed with unrounded values

[1)] based on NO_3-N-production; - = inhibition; + = stimulation

In a separate study the reference item Dinoterb caused stimulations of nitrogen transformation of +59.9 %, +216.3 % and +238.5 % at 6.80, 13.60 and 27.20 mg Dinoterb per kg soil dry weight, respectively, determined 28 days after application (time interval 14-28). For details see **Błąd! Nie można odnaleźć źródła odwołania.**, page **Błąd! Nie zdefiniowano zakładki.**.

CONCLUSION

The test item GLOB1310aH (tested at 3.8 mg/kg soil dry weight and 19 mg/kg soil dry weight) caused no adverse effects (deviation from control <25 %, OECD 216) on soil nitrogen transformation (measured as NO_3-N-production) at the end of the 28-day incubation period.

Comments of zRMS:	The study was conducted to OECD guideline 216 and according to the principles of GLP. All the validity criterion are met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.5
Report	Soil micro-organisms: Nitrogen and carbon transformation test with FOE sulfonic acid (OECD 216 and OECD 217, January 2000). Servajean I. 2014. Study 14-99-006-ES
Guideline(s):	Yes (OECD 216 and 217)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

Materials and Methods

Test material: FOE sulfonic acid.

Method: Soil collection: the soil was collected in April 2014 according to the standardized method ISO 10381-6. The soil was sieved through a 2 mm aperture size.

Test system: For the nitrogen mineralization, the soil was divided into distinct portions corresponding to 500 g dry soiland amended with 2.5 g of powdered lucerne-grass-green meal and moistened to 37% of its total water holding capacity. As the soil was freshly collected, the soil fractions were not pre-incubated.. For the carbon mineralization, the soil was divided into distinct portions corresponding to 700.0 g dry soil and moistened to 37% of its total water holding capacity. As the soil was freshly collected, the soil fractions were not pre-incubated.

Test concentrations and number of replicates: Two distinct concentrations were used. The lower concentration 1x, was twice the expected concentration in soil. The upper concentration was 10 times that concentration. The two concentrations were 0.52 and 5.2 mg/kg dry soil.

The reference substance, Fumical (Metam-sodium 510 g/l), was tested at the concentration of 1.3 ml/kg dry soil.

Three replicate bulk samples were used for every treatment and concentrations. In every case, the soil fractions were thoroughly mixed after the treatment application and placed in the dark at 20°C.

Measurements and assessments:
Nitrogen mineralization:
The nitrate formation was determined within 6 hours of addition of the pesticide, and then on day 7, 14 and 28 of the test on accuracy weighed samples of 10 g of soil. Nitrates were extracted with 40 ml of a 50 mg/L ethylene diamine solution added with 0.1% H_2SO_4. Based on the results, the nitrate formation was also assessed on day 42, 56 and 70 using the same method.

Carbon mineralization:
The carbon mineralization was determined within 6 hours of addition of the pesticide, and then on day 7, 14 and 28 of the test on accuracy weighed samples of 50 g of soil. Soil samples were mixed with 0.3% w/w glucose ground with quartz sand so as to induce an immediate respiratory response. The respiration rate was assessed from the oxygen consumed by the glucose amended soil samples using an incubation system combined with a manometric oxygen measurement. This consumption of oxygen was measured for 24 consecutive hours and was initiated within 1 hour after glucose supplement.

Results

A. Nitrogen mineralization:

The results of the nitrogen mineralization test are reported in the table below

Treatment	$N\text{-}NO_3$ mg/kg dry soil (mean of 3 replicates)		
	D_0 + 7 d	D_0 + 14 d	D_0 + 28 d
Control	-6.28 ± -2.8%	5.34 ± 7.4%	27.44 ± 3.8%
FOE sulfonic acid 0.52 mg/kg	-6.29 ± -0.7%	5.22 ± 20.7%	29.02 ± 7.4%
FOE sulfonic acid 5.2 mg/kg	-6.82 ± -1.8%	5.14 ± 17.9%	27.90 ± 5.2%
Fumical	2.88 ± -5.5% -	1.86 ± -75.8%	29.96 ± 4.9%

A slight depletion was observed on day 7, as it is classically the case when the soils are not pre-incubated. On days 14 and 28 the production of nitrate was similar to that of the controls for the two test item treatments (5%-confidence level).

As a comparison, the production of nitrate was significantly altered in the Fumical treated fractions as compared to the controls: the formation of nitrate was reduced by more than 50 % on day 14 and then restored on day 28.

B. Carbon mineralization:

The results of the carbon mineralization test are presented in the table below.

Treatment	mg O_2/kg dry soil (mean of 3 replicates +/ SD (in %))			
	D0	D0 + 7 d	D0 + 14 d	D0 + 28 d
Control	14.00 ± 3.3%	11.14 ± 3.2%	11.65 ± 4.6%	15.73 ± 5.6%
FOE sulfonic acid 0.52 mg/kg	13.49 ± 2.3%	10.83 ± 1.6%	11.44 ±4.1%	15.33 ±4.0%
FOE sulfonic acid 5.2 mg/kg	13.49 ± 2.3%	10.93 ± 1.6%	11.55 ± 4.1%	15.22 ± 1.2%
Fumical	6.44±4.8%	11.44 ± 8.6%	11.55 ± 5.5%	13.18 ± 2.3%

The induced respiration was sustained in the water controls throughout the incubation period. Replicate measured values within the water controls deviated by less than 15% of mean value at each sampling time, as required.

At each sampling time the induced respiration was considered as similar to that of the controls for the two test item treatments (5% confidence level).
As a comparison the induced respiration was reduced by 54 % for the reference item treatment soon after the treatment application. As a comparison the induced respiration was reduced by 55 % for the reference item treatment soon after the treatment application.

Conclusion:

At each sampling time the induced respiration was considered as similar to that of the controls for the two test item treatments (5% confidence level). On days 14 and 28 the production of nitrate was similar to that of the controls for the two test item treatments (5%-confidence level).

Comments of zRMS:	The study was conducted to OECD guideline 216 and according to the principles of GLP. All the validity criterion are met. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.5
Report	Soil micro-organisms: Nitrogen and carbon transformation test with FOE oxalate (OECD 216 and OECD 217, January 2000). Servajean I. 2014. Study 14-99-007-ES
Guideline(s):	Yes (OECD 216 and 217)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

Materials and Methods

Test material: FOE oxalate.

Method: Soil collection: the soil was collected in April 2014 according to the standardized method ISO 10381-6. The soil was sieved through a 2 mm aperture size.

Test system: For the nitrogen mineralization, the soil was divided into distinct portions corresponding to 500 g dry soil and amended with 2.5 g of powdered lucerne-grass-green meal and moistened to 37% of its total water holding capacity. As the soil was freshly collected, the soil fractions were not pre-incubated.. For the carbon mineralization, the soil was divided into distinct portions corresponding to 700.0 g dry soil and moistened to 37% of its total water holding capacity. As the soil was freshly collected, the soil fractions were not pre-incubated.

Test concentrations and number of replicates: Two distinct concentrations were used. The lower concentration 1x, was twice the expected concentration in soil. The upper concentration was 10 times that concentration. The two concentrations were 0.16 and 1.6 mg/kg dry soil. The reference substance, Fumical (Metam-sodium 510 g/l), was tested at the concentration of 1.3 ml/kg dry soil. Three replicate bulk samples were used for every treatment and concentrations. In every case, the soil fractions were thoroughly mixed after the treatment application and placed in the dark at 20°C.

Measurements and assessments:
Nitrogen mineralization:
The nitrate formation was determined within 6 hours of addition of the pesticide, and then on day 7, 14 and 28 of the test on accuracy weighed samples of 10 g of soil. Nitrates were extracted with 40 ml of a 50 mg/L ethylene diamine solution added with 0.1% H_2SO_4. Based on the results, the nitrate formation was also assessed on day 42, 56 and 70 using the same method.

Carbon mineralization:
The carbon mineralization was determined within 6 hours of addition of the pesticide, and then on day 7, 14 and 28 of the test on accuracy weighed samples of 50 g of soil. Soil samples were mixed with 0.3%

w/w glucose ground with quartz sand so as to induce an immediate respiratory response. The respiration rate was assessed from the oxygen consumed by the glucose amended soil samples using an incubation system combined with a manometric oxygen measurement. This consumption of oxygen was measured for 24 consecutive hours and was initiated within 1 hour after glucose supplement.

Results

A. Nitrogen mineralization:

The results of the nitrogen mineralization test are reported in the table below

Treatment	$N\text{-}NO_3$ mg/kg dry soil (mean of 3 replicates)		
	D_0 + 7 d	D_0 + 14 d	D_0 + 28 d
Control	-6.36 ± -2.8%	5.26 ± 7.5%	27.36 ± 3.8%
FOE oxalate 0.16 mg/kg	-6.33 ± -2.4%	4.33 ± 19.7%	26.20 ± 3.0%
FOE oxalate 1.6 mg/kg	-5.67 ± -3.2%	4.97 ± 45.6%	26.86 ± 12.2%
Fumical	-2.96 ± -5.3% -	1.93 ± -72.7%	29.88 ± 4.9%

A slight depletion was observed on day 7, as it is classically the case when the soils are not pre-incubated. The 1.60 mg/kg test item treatment was not as depleted as the controls and the difference was significant. On days 14 and 28 the production of nitrate was similar to that of the controls for the two test item treatments (5%-confidence level).

As a comparison, the production of nitrate was significantly altered in the Fumical treated fractions as compared to the controls: the formation of nitrate was reduced by more than 50 % on day 14 and then restored on day 28.

B. Carbon mineralization:

The results of the carbon mineralization test are presented in the table below

Treatment	mg O_2/kg dry soil (mean of 3 replicates +/ SD (in %))			
	D0	D0 + 7 d	D0 + 14 d	D0 + 28 d
Control	14.00 ± 3.3%	11.14 ± 3.2%	11.65 ± 4.6%	15.73 ± 5.6%
FOE oxalate 0.16 mg/kg	14.41 ±4.3%	10.52±3.4%	11.14±3.2%	14.51 ±3.2%
FOE oxalate 1.6 mg/kg	13.38 ± 3.5%	10.11 ± 3.0%	10.83 ± 1.6%	13.49 ± 3.9%
Fumical	6.44 ±4.8%	11.44 ± 8.6%	11.55 ± 5.5%	13.18 ± 2.3%

The induced respiration was sustained in the water controls throughout the incubation period.
Replicate measured values within the water controls deviated by less than 15% of mean value at each sampling time, as required.
At each sampling time the induced respiration was considered as similar to that of the controls for the two test item treatments (5% confidence level).
As a comparison the induced respiration was reduced by 54 % for the reference item treatment soon after the treatment application.

Conclusion:
At each sampling time the induced respiration was considered as similar to that of the controls for the two test item treatments (5% confidence level). On days 14 and 28 the production of nitrate was similar to that of the controls for the two test item treatments (5%-confidence level).

A 2.6 KCP 10.6 Effects on terrestrial non-target higher plants

A 2.6.1 KCP 10.6.1 Summary of screening data

A 2.6.2 KCP 10.6.2 Testing on non-target plants

Comments of zRMS:	The study was conducted to OECD guideline 227 and according to the principles of GLP. All the validity criterion are met. Seedling emergence: ≥ 70 % (actual 80 - 100 %). For control group: mean plant survival for the duration of the study: ≥ 90 % (actual 100 %), plants do not exhibit visible phytotoxic effects in the control and the plants exhibit only normal variation in growth and morphology for that particular species, environmental conditions for a particular species are identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source. The study is considered to be reliable and suitable for the risk assessment.

Reference:	KCP 10.6.2
Report	Effects of GLOB1310aH on vegetative vigour of ten non-target terrestrial plant species under greenhouse conditions. Friedemann. A, 2021a. Study No. 21 46 PVV 0001
Guideline(s):	Yes (OECD TG 227 (2006))
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

SUMMARY

Study title:	Effects of GLOB1310aH on vegetative vigour of ten non-target terrestrial plant species under greenhouse conditions
Guideline:	OECD 227 (2006)
Test item:	GLOB1310aH
Batch no.:	GLO-20F-2306A

Active ingredient/content:

	nominal	analysed
Aclonifen:	540 g/L	543.7 g/L
Flufenacet:	60 g/L	61.55 g/L

Test species:	oilseed rape (*Brassica napus*), cucumber (*Cucumis sativus),* lettuce (*Lactuca sativa*), soybean (*Glycine max*), ryegrass (*Lolium perenne*), great millet *(Sorghum bicolor*), sugar beet (*Beta vulgaris*), flax (*Linum usitatissimum*), onion (*Allium cepa*), oat (*Avena sativa*)
Application rates:	0.0 and 8.3, 14.4, 24.9, 43.1, 74.6, 129.1, 223.4, 386.5, 668.6 and 1156.7 mL GLOB1310aH/ha in 200 L water/ha
Experimental phase:	26.01.2021 – 22.02.2021

Material and methods:

Potential adverse effects of the test item GLOB1310aH to ten terrestrial plant species were examined in comparison with a water control under greenhouse conditions. During the test the climate conditions were as follows: air temperature 14.6 – 31.6 °C, relative humidity 41.3 – 71.1 %, mean light intensity 386 - 424 µmol/m²/s (during 16 hour photoperiod).

In the test GLOB1310aH was applied at BBCH stage 12-14 at application rates of 8.3, 14.4, 24.9, 43.1, 74.6, 129.1, 223.4, 386.5, 668.6 and 1156.7 mL test item/ha. The test solution was sprayed once onto the plants in an automatic application cabin at a spray volume equivalent to 200 L/ha. The control and the highest test item solution were sampled directly after preparation and immediately before the applications. The recoveries were 98 % of nominal Aclonifen and 99 % of nominal Flufenacet, so the nominal concentrations of the test solutions could be analytically confirmed.

During the observation period of 21 days after treatment (DAT), the plants were assessed weekly for plant survival, growth stage and visual injury. Endpoints observed 21 DAT were plant survival, plant height, biomass and visible injury and growth stage.

Statistical analysis of data was performed using the software ToxRat Professional (Version 3.3.0).

All validity criteria were met:
- Seedling emergence: ≥ 70 % (actual 80 - 100 %)

For control group:
- Mean plant survival for the duration of the study: ≥ 90 % (actual 100 %)
- Plants do not exhibit visible phytotoxic effects in the control and the plants exhibit only normal variation in growth and morphology for that particular species
- Environmental conditions for a particular species are identical and growing media contained the same amount of soil matrix, support media, or substrate from the same source

Results

The effects of GLOB1310aH after application at BBCH stage 12-14 on different plant species were examined at nominal application rates of 8.3, 14.4, 24.9, 43.1, 74.6, 129.1, 223.4, 386.5, 668.6 and 1156.7 mL test item/ha in 200 L water/ha under greenhouse conditions. The test endpoints were plant survival, plant height, biomass and visual injury on day 21 after application.

Plant survival
No significant reduction of plant survival could be detected after pre-emergence application of GLOB1310aH for all tested plant species except lettuce and flax.

Effects of GLOB1310aH on plant survival 21 DAT [% compared to control]

Test Species	Application rates [mL GLOB1310aH/ha]										
	0.0	**8.3**	**14.4**	**24.9**	**43.1**	**74.6**	**129.1**	**223.4**	**386.5**	**668.6**	**1156.7**
Błąd! Nie można odnaleźć źródła odwołania.	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	100.0	--	--	100.0	100.0	100.0	100.0	80.0**	5.0**	--	--
Błąd! Nie można odnaleźć źródła	100.0	--	--	--	100.0	100.0	100.0	100.0	100.0	--	--

odwołania.											
Błąd! Nie można odnaleźć źródła odwołania.	100.0	--	--	--	100.0	100.0	100.0	100.0	100.0	--	--
Błąd! Nie można odnaleźć źródła odwołania.	100.0	--	--	--	100.0	100.0	100.0	100.0	100.0	--	--
Błąd! Nie można odnaleźć źródła odwołania.	100.0	--	--	--	--	--	100.0	100.0	100.0	100.0	100.0
Błąd! Nie można odnaleźć źródła odwołania.	100.0	--	--	--	--	--	100.0	100.0	100.0	90.0*	50.0*
Błąd! Nie można odnaleźć źródła odwołania.	100.0	--	--	--	--	--	100.0	100.0	100.0	100.0	100.0
Błąd! Nie można odnaleźć źródła odwołania.	100.0	--	--	--	--	--	100.0	100.0	100.0	100.0	100.0

DAT days after treatment
statistical calculations based on number of surviving plants per replicate:
* significantly different to control (Step-down Cochran-Armitage Test (one-sided greater, α=0.05))
** significantly different to control (Step-down Rao-Scott-Cochran-Armitage Test (one-sided greater, α=0.05))

The NOER for plant survival was 129.1 mL GLOB1310aH/ha for lettuce, ≥ 223.4 mL GLOB1310aH/ha for oilseed rape and cucumber, 386.5 mL GLOB1310aH/ha for flax, ≥ 386.5 mL GLOB1310aH/ha for soybean, ryegrass and great millet, ≥ 1156.7 mL GLOB1310aH/ha for sugar beet, onion and oat.
A rate-response for plant survival could be found for lettuce and flax with ER_{50} values of 269.0 and 1149.0 mL GLOB1310aH/ha respectively.

NOER, ER_{25} and ER_{50} for plant survival 21 DAT [mL GLOB1310aH/ha]

Test species	NOER	ER_{25}	ER_{50}
Błąd! Nie można odnaleźć źródła odwołania.	≥ 223.4	> 223.4	> 223.4
Błąd! Nie można odnaleźć źródła odwołania.	≥ 223.4	> 223.4	> 223.4
Błąd! Nie można odnaleźć źródła odwołania.	129.1	232.0	269.0
Błąd! Nie można odnaleźć źródła odwołania.	≥ 386.5	> 386.5	> 386.5
Błąd! Nie można odnaleźć źródła odwołania.	≥ 386.5	> 386.5	> 386.5
Błąd! Nie można odnaleźć źródła odwołania.	≥ 386.5	> 386.5	> 386.5
Błąd! Nie można odnaleźć źródła odwołania.	≥ 1156.7	> 1156.7	> 1156.7
Błąd! Nie można odnaleźć źródła odwołania.	386.5	875.6	1149.0
Błąd! Nie można odnaleźć	≥ 1156.7	> 1156.7	> 1156.7

źródła odwołania.			
Błąd! Nie można odnaleźć źródła odwołania.	≥ 1156.7	> 1156.7	> 1156.7

DAT days after treatment NOER no observed effect rate ER_X X% effect rate

Growth stage

The application of GLOB1310aH at BBCH stage 12-14 caused no visible effects on growth stage of soybean, great millet and onion. The development of growth stage of oilseed rape, cucumber, lettuce, ryegrass, sugar beet, flax and oat were delayed.

Effects of GLOB1310aH on growth stage 21 DAT (minimum to maximum of BBCH stage)

Test Species	Application rates [mL GLOB1310aH/ha]										
	0.0	**8.3**	**14.4**	**24.9**	**43.1**	**74.6**	**129.1**	**223.4**	**386.5**	**668.6**	**1156.7**
Błąd! Nie można odnaleźć źródła odwołania.	19	19	19	19	19	19	19	17-19	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	63	63	63	63	63	63	63	61-63	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	19	--	--	19	19	18-19	16-18	13-17	13	--	--
Błąd! Nie można odnaleźć źródła odwołania.	63	--	--	--	63	63	63	63	63	--	--
Błąd! Nie można odnaleźć źródła odwołania.	29	--	--	--	29	29	29	29	12-29	--	--
Błąd! Nie można odnaleźć źródła odwołania.	30	--	--	--	30	30	30	30	30	--	--
Błąd! Nie można odnaleźć źródła odwołania.	18-19	--	--	--	--	--	17-18	17-18	17-18	17-18	15-18
Błąd! Nie można odnaleźć źródła odwołania.	24/39	--	--	--	--	--	22-24	22-24	22/39	33-22	31-21
Błąd! Nie można odnaleźć źródła odwołania.	41	--	--	--	--	--	41	41	41	41	41
Błąd! Nie można odnaleźć źródła	31	--	--	--	--	--	31	31	31	30-31	13-30

odwołania.											

DAT days after treatment

Phytotoxicity

The application of GLOB1310aH at BBCH stage 12-14 caused visible phytotoxic effects on all tested plant species. Up to the respective highest tested rate slight symptoms were seen 21 DAT in great millet and onion, moderate symptoms in oilseed rape, soybean and ryegrass, severe symptoms in cucumber and oat and moribund plants were seen in lettuce, sugar beet and flax.

Effects of GLOB1310aH on phytotoxicity 21 DAT [EPPO rating in %]

Test	**Application rates [mL GLOB1310aH/ha]**										
Species	**0.0**	**8.3**	**14.4**	**24.9**	**43.1**	**74.6**	**129.1**	**223.4**	**386.5**	**668.6**	**1156.7**
Błąd! Nie można odnaleźć źródła odwołania.	0.0	10.0	10.0	10.0	12.0	20.0	20.0	33.0	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	10.0	17.0	20.0	26.0	30.0	37.0	44.0	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	0.0	14.0	20.0	35.0	84.0	99.0	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	10.0	10.0	20.0	20.0	30.0	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	0.0	0.0	0.0	10.0	24.0	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	0.0	10.0	10.0	10.0	10.0	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	29.0	30.0	34.0	44.0	64.0
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	14.0	20.0	50.0	70.0	90.0
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	0.0	0.0	6.0	10.0	20.0
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	0.0	10.0	10.0	32.0	56.0

DAT days after treatment

Plant height

No significant reduction of plant height could be detected for cucumber, soybean, great millet and onion after application of GLOB1310aH at BBCH stage 12-14. In oilseed rape, lettuce, ryegrass, sugar beet, flax and oat the plant height was reduced significantly.

Effects of GLOB1310aH on plant height 21 DAT [% inhibition]

Test	Application rates [mL GLOB1310aH/ha]										
Species	0.0	8.3	14.4	24.9	43.1	74.6	129.1	223.4	386.5	668.6	1156.7
Błąd! Nie można odnaleźć źródła odwołania.	0.0	1.9	3.2	4.4	4.5	4.9*	7.6*	12.5*	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	-8.8	-8.3	-10.3	3.8	-7.2	-3.7	7.7	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	-1.8	-2.1	3.0	7.6**	46.5**	97.3**	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	-5.3	-4.4	-3.3	-3.3	1.5	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	-7.7	-4.6	-0.1	-4.7	14.2*	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	-3.9	-3.1	-4.0	-2.5	-5.9	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	18.9*	15.6*	22.8*	20.7*	22.8*
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	17.5**	25.9**	41.8**	60.3**	83.7**
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	4.1	3.9	0.7	0.5	3.0
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	1.9	4.8	-0.2	10.3*	16.3*

DAT days after treatment
statistical calculations based on plant height in cm per replicate:
* significantly different to control (Williams Multiple sequential t-test (one-sided smaller, α=0.05))

** significantly different to control (Multiple sequentially-rejective Welch-t-test after Bonferroni-Holm (one-sided smaller, α=0.05))

The NOER for plant height reduction for cucumber, soybean, great millet and onion appears to be higher than or equal the respective highest tested rate. The NOER for oat was 386.5 mL GLOB1310aH/ha, for ryegrass 223.4 mL GLOB1310aH/ha, for lettuce 74.6 mL GLOB1310aH/ha and for oilseed rape 43.1 mL GLOB1310aH/ha. The NOER for sugar beet and flax could not be determined and was < 129.1 mL GLOB1310aH/ha.
A rate-response for plant height reduction could be found for lettuce and flax with ER_{50} values of 227.1 and 459.7 mL GLOB1310aH/ha respectively.

NOER, ER_{25} and ER_{50} for plant height reduction 21 DAT [mL GLOB1310aH/ha]

Test species	NOER	ER_{25}	ER_{50}
Błąd! Nie można odnaleźć źródła odwołania.	43.1	> 223.4	> 223.4
Błąd! Nie można odnaleźć źródła odwołania.	≥ 223.4	> 223.4	> 223.4
Błąd! Nie można odnaleźć źródła odwołania.	74.6	181.6	227.1
Błąd! Nie można odnaleźć źródła odwołania.	≥ 386.5	> 386.5	> 386.5
Błąd! Nie można odnaleźć źródła odwołania.	223.4	> 386.5	> 386.5
Błąd! Nie można odnaleźć źródła odwołania.	≥ 386.5	> 386.5	> 386.5
Błąd! Nie można odnaleźć źródła odwołania.	< 129.1	n.d.	n.d.
Błąd! Nie można odnaleźć źródła odwołania.	< 129.1	214.9	459.7
Błąd! Nie można odnaleźć źródła odwołania.	≥ 1156.7	> 1156.7	> 1156.7
Błąd! Nie można odnaleźć źródła odwołania.	386.5	> 1156.7	> 1156.7

DAT days after treatment NOER no observed effect rate ER_X X% effect rate
n.d.: not determined due to mathematical reasons or inappropriate data

Biomass
The biomass was reduced significantly for all tested plant species after application of GLOB1310aH at BBCH stage 12-14 except great millet.

Effects of GLOB1310aH on biomass 21 DAT [% inhibition]

Test Species	Application rates [mL GLOB1310aH/ha]										
	0.0	**8.3**	**14.4**	**24.9**	**43.1**	**74.6**	**129.1**	**223.4**	**386.5**	**668.6**	**1156.7**
Błąd! Nie można odnaleźć źródła odwołania.	0.0	1.3	0.5	-1.1	8.3	15.7***	18.6***	45.4***	--	--	--
Błąd! Nie można	0.0	4.4	8.6	6.4	18.6*	8.4*	14.9*	24.7*	--	--	--

odnaleźć źródła odwołania.											
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	12.4**	21.1**	38.8**	71.6**	92.9**	99.9**	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	4.3	0.6	8.1***	16.0***	21.5***	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	5.6	4.7	7.5	14.5*	46.0*	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	-0.7	3.0	2.6	6.3	-1.4	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	45.5**	46.1**	55.9**	58.7**	64.9**
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	39.7**	49.0**	65.5**	84.6**	95.8**
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	8.9	7.7	13.0	5.5	18.2*
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	9.0*	19.8*	26.7*	54.1*	66.0*

DAT days after treatment
statistical calculations based on biomass in g per replicate:
* significantly different to control (Williams Multiple Sequential t-test (one-sided smaller, α=0.05))
** significantly different to control (Multiple sequentially-rejective Welch-t-test after Bonferroni-Holm (one-sided smaller, α=0.05))
*** significantly different to control (Step-down Jonckheere-Terpstra test (one-sided smaller, α=0.05))

The NOER of biomass reduction for onion was 668.6 mL GLOB1310aH/ha, for great millet ≥ 386.5 mL GLOB1310aH/ha, for ryegrass 129.1 mL GLOB1310aH/ha, 74.6 mL GLOB1310aH/ha for soybean, 43.1 mL GLOB1310aH/ha for oilseed rape and 24.9 mL GLOB1310aH/ha for cucumber.
The NOER for lettuce, sugar beet, flax and oat could not be determined and was < 129.1 mL GLOB1310aH/ha for sugar beet, flax and oat and < 24.9 mL GLOB1310aH/ha for lettuce.
A rate-response for biomass reduction could be found for oilseed rape, lettuce, ryegrass, sugar beet, flax and oat. For oilseed rape and ryegrass ER_{25} values could be calculated with 132.6 and 295.1 mL GLOB1310aH/ha. The ER_{50} for sugar beet, flax and oat ranged between 213.9 and 675.7 mL GLOB1310aH/ha. The lowest ER_{50} was found for lettuce with 83.7 mL GLOB1310aH/ha.

NOER, ER_{25} and ER_{50} for biomass reduction 21 DAT [mL GLOB1310aH/ha]

Test species	NOER	ER_{25}	ER_{50}
Błąd! Nie można odnaleźć źródła	43.1	132.6	> 223.4

odwołania.			
Błąd! Nie można odnaleźć źródła odwołania.	24.9	> 223.4	> 223.4
Błąd! Nie można odnaleźć źródła odwołania.	< 24.9	49.4	83.7
Błąd! Nie można odnaleźć źródła odwołania.	74.6	> 386.5	> 386.5
Błąd! Nie można odnaleźć źródła odwołania.	129.1	295.1	> 386.5
Błąd! Nie można odnaleźć źródła odwołania.	≥ 386.5	> 386.5	> 386.5
Błąd! Nie można odnaleźć źródła odwołania.	< 129.1	n.d.	249.5
Błąd! Nie można odnaleźć źródła odwołania.	< 129.1	n.d.	213.9
Błąd! Nie można odnaleźć źródła odwołania.	668.6	> 1156.7	> 1156.7
Błąd! Nie można odnaleźć źródła odwołania.	< 129.1	306.8	675.7

DAT days after treatment NOER no observed effect rate ERX X% effect rate
n.d.: not determined either due to mathematical reasons or value is beyond the tested concentrations by more than factor 1000.

Conclusion

The effects of GLOB1310aH after application at BBCH stage 12-14 on ten terrestrial plant species were examined at nominal application rates of 8.3, 14.4, 24.9, 43.1, 74.6, 129.1, 223.4, 386.5, 668.6 and 1156.7 mL test item/ha in 200 L water/ha under greenhouse conditions. The test endpoints for oilseed rape, cucumber, lettuce, soybean, ryegrass, great millet, sugar beet, flax, onion and oat were plant survival (mortality), plant height, biomass and visual phytotoxicity 21 days after 50 % emergence in the control group (DAE).

All validity criteria of the vegetative vigour study were met. The recoveries of the active ingredients in the spray solution specimen of Treatment 11 were 98 % for Aclonifen and 99 % for Flufenacet. In the control specimens no active ingredient above 30 % of the LOQ was detected. Thus, the concentrations in the specimen of the biological part of the study were verified. During the study, the climate conditions were mainly kept and plants of untreated control looked healthy and grew normally. Thus, the study was valid.

No significant reduction of plant survival could be detected. The NOER for plant survival was 129.1 mL GLOB1310aH/ha for lettuce, ≥ 223.4 mL GLOB1310aH/ha for oilseed rape and cucumber, 386.5 mL GLOB1310aH/ha for flax, ≥ 386.5 mL GLOB1310aH/ha for soybean, ryegrass and great millet, ≥ 1156.7 mL GLOB1310aH/ha for sugar beet, onion and oat.
A rate-response for plant survival could be found for lettuce and flax with ER_{50} values of 269.0 and 1149.0 mL GLOB1310aH/ha respectively.

The application of GLOB1310aH at BBCH stage 12-14 caused no visible effects 21 DAT on growth stage of soybean, great millet and onion. The development of growth stage of oilseed rape, cucumber, lettuce, ryegrass, sugar beet, flax and oat were delayed.

Visible phytotoxic effects were seen on all tested plant species. Up to the respective highest tested rate slight symptoms were seen 21 DAT in great millet and onion, moderate symptoms in oilseed rape, soybean and ryegrass, severe symptoms in cucumber and oat and moribund plants were seen in lettuce, sugar beet and flax.

No significant reduction of plant height could be detected for cucumber, soybean, great millet and onion. In oilseed rape, lettuce, ryegrass, sugar beet, flax and oat the plant height was reduced significantly. The NOER for plant height reduction for cucumber, soybean, great millet and onion appears to be higher than or equal the respective highest tested rate. The NOER for oat was 386.5 mL GLOB1310aH/ha, for ryegrass 223.4 mL GLOB1310aH/ha, for lettuce 74.6 mL GLOB1310aH/ha and for oilseed rape 43.1 mL GLOB1310aH/ha. The NOER for sugar beet and flax could not be determined and was < 129.1 mL GLOB1310aH/ha.
A rate-response for plant height reduction could be found for lettuce and flax with ER_{50} values of 227.1 and 459.7 mL GLOB1310aH/ha respectively.

The biomass was reduced significantly for all tested plant species except great millet. The NOER of biomass reduction for onion was 668.6 mL GLOB1310aH/ha, for great millet ≥ 386.5 mL GLOB1310aH/ha, for ryegrass 129.1 mL GLOB1310aH/ha, 74.6 mL GLOB1310aH/ha for soybean, 43.1 mL GLOB1310aH/ha for oilseed rape and 24.9 mL GLOB1310aH/ha for cucumber.
The NOER for lettuce, sugar beet, flax and oat could not be determined and was < 129.1 mL GLOB1310aH/ha for sugar beet, flax and oat and < 24.9 mL GLOB1310aH/ha for lettuce.
A rate-response for biomass reduction could be found for oilseed rape, lettuce, ryegrass, sugar beet, flax and oat. For oilseed rape and ryegrass ER_{25} values could be calculated with 132.6 and 295.1 mL GLOB1310aH/ha. The ER_{50} for sugar beet, flax and oat ranged between 213.9 and 675.7 mL GLOB1310aH/ha. The lowest ER_{50} was found for lettuce with 83.7 mL GLOB1310aH/ha.

Comments of zRMS:	The study was conducted to OECD guideline 227 and according to the principles of GLP. All the validity criterion are met. Seedling emergence in the control: ≥ 70 % (actual 86 - 100 %), mean survival of emerged control seedlings: ≥90 % (actual 100 %), no visible phytotoxic effects were seen in the control and the plants exhibit only normal variation in growth and morphology for the test species, environmental conditions were identical and growing media contained the same amount of soil from the same source. The study is considered to be reliable and suitable for the risk assessment.-

Reference:	KCP 10.6.2
Report	Effects of GLOB1310aH on seedling emergence and seedling growth of ten non-target terrestrial plant species under greenhouse conditions. Friedemann. A, 2021b. Study No. 21 46 PSE 0001
Guideline(s):	Yes (OECD TG 208 (2006))
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not a vertebrate study

SUMMARY

Study title:	Effects of GLOB1310aH on vegetative vigour of ten non-target terrestrial plant species under greenhouse conditions
Guideline:	OECD 208 (2006)
Test item:	GLOB1310aH
Batch no.:	GLO-20F-2306A

Active ingredient/content:	nominal	analysed
Aclonifen:	540 g/L	543.7 g/L
Flufenacet:	60 g/L	61.55 g/L

Test species:	lettuce (*Lactuca sativa*), oilseed rape (*Brassica napus),* ryegrass (*Lolium perenne*), sugar beet (*Beta vulgaris*), radishes (*Raphanus sativus*), tomato *(Solanum lycopersicum*), onion (*Allium cepa*), cucumber (*Cucumis sativus*), oat (*Avena sativa*), barley (*Hordeum vulgare*)
Application rates:	0.0 and 4.3, 7.5, 13.0, 22.8, 39.9, 69.9, 122.3, 214.1, 374.7, 655.7, 1147.4 and 2008.0 mL GLOB1310aH/ha in 200 L water/ha
Experimental phase:	06.11.2020 – 04.12.2020

Material and methods:

Potential adverse effects of the test item GLOB1310aH to ten terrestrial plant species were examined in comparison with a water control under greenhouse conditions. During the test the climate conditions were as follows: air temperature 15.8 – 28.5 °C, relative humidity 39.2 – 69.6 %, mean light intensity 433 µmol/m²/s (during 16 hour photoperiod).

In the test GLOB1310aH was applied after sowing at application rates of 8.3, 14.4, 24.9, 43.1, 74.6, 129.1, 223.4, 386.5, 668.6 and 1156.7 mL test item/ha. The test solution was sprayed once onto the soil surface in an automatic application cabin at a spray volume equivalent to 200 L/ha. The control and the highest test item solution were sampled directly after preparation and immediately before application. The recoveries were 98 % of nominal Aclonifen and 98 % of nominal Flufenacet, so the nominal concentrations of the test solutions could be analytically confirmed.

During the observation period of 21 days after 50 % of the control plants had emerged (DAE), the plants were assessed weekly for seedling emergence, plant survival, growth stage and visual injury. Endpoints observed 21 DAE were seedling emergence, plant survival, plant height, biomass and visible injury and growth stage.

Statistical analysis of data was performed using the software ToxRat Professional (Version 3.3.0).

All validity criteria were met:
- Seedling emergence in the control: ≥ 70 % (actual 86 - 100 %)
- Mean survival of emerged control seedlings: ≥ 90 % (actual 100 %)
- No visible phytotoxic effects were seen in the control and the plants exhibit only normal variation in growth and morphology for the test species
- Environmental conditions were identical and growing media contained the same amount of soil from the same source

Results

The effects of GLOB1310aH after pre-emergence application on different plant species were examined at nominal application rates of 8.3, 14.4, 24.9, 43.1, 74.6, 129.1, 223.4, 386.5, 668.6 and 1156.7 mL test item/ha in 200 L water/ha under greenhouse conditions. The test endpoints were seedling emergence, plant survival, plant height, biomass and visual injury on day 21 after 50 % emergence in the control.

Seedling emergence and plant survival
No significant reduction of seedling emergence and plant survival could be detected after pre-emergence application of GLOB1310aH for all tested plant species.

Effects of GLOB1310aH on seedling emergence and plant survival 21 DAE [% compared to control]

Test	Application rates [mL GLOB1310aH/ha]												
Species	0.0	4.3	7.5	13.0	22.8	39.9	69.9	122.3	214.1	374.7	655.7	1147.4	2008.0
Błąd! Nie można odnaleźć źródła													

odwołania.													
Emergence	100.0	100.0	100.0	100.0	100.0	100.0	100.0	--	--	--	--	--	--
Survival	100.0	100.0	100.0	100.0	100.0	100.0	100.0	--	--	--	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.													
Emergence	100.0	--	--	100.0	100.0	100.0	100.0	100.0	100.0	--	--	--	--
Survival	100.0	--	--	100.0	100.0	100.0	100.0	100.0	100.0	--	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.													
Emergence	100.0	--	--	104.3	100.0	100.0	100.0	104.3	104.3	--	--	--	--
Survival	100.0	--	--	100.0	100.0	100.0	100.0	100.0	100.0	--	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.													
Emergence	100.0	--	--	--	--	100.0	100.0	100.0	100.0	100.0	--	--	--
Survival	100.0	--	--	--	--	100.0	100.0	100.0	100.0	100.0	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.													
Emergence	100.0	--	--	--	--	--	100.0	88.9	88.9	94.4	94.4	--	--
Survival	100.0	--	--	--	--	--	100.0	100.0	100.0	100.0	82.4	--	--
Błąd! Nie można odnaleźć źródła odwołania.													
Emergence	100.0	--	--	--	--	--	100.0	100.0	100.0	100.0	85.7	--	--
Survival	100.0	--	--	--	--	--	100.0	100.0	100.0	100.0	100.0	--	--
Błąd! Nie można odnaleźć źródła odwołania.													
Emergence	100.0	--	--	--	--	--	104.8	100.0	100.0	104.8	90.5	--	--
Survival	100.0	--	--	--	--	--	100.0	100.0	100.0	100.0	100.0	--	--
Błąd! Nie można odnaleźć źródła odwołania.													
Emergence	100.0	--	--	--	--	--	100.0	100.0	100.0	100.0	100.0	--	--
Survival	100.0	--	--	--	--	--	100.0	100.0	100.0	100.0	100.0	--	--
Błąd! Nie można odnaleźć źródła odwołania.													
Emergence	100.0	--	--	--	--	--	--	100.0	104.3	104.3	104.3	100.0	--
Survival	100.0	--	--	--	--	--	--	100.0	100.0	100.0	100.0	100.0	--
Błąd! Nie można odnaleźć źródła odwołania.													
Emergence	100.0	--	--	--	--	--	--	--	100.0	100.0	100.0	100.0	100.0
Survival	100.0	--	--	--	--	--	--	--	100.0	100.0	100.0	100.0	100.0

DAE days after 50 % emergence in the control group

The NOER for seedling emergence and plant survival was ≥ 69.9 mL GLOB1310aH/ha for lettuce, ≥ 214.1 mL GLOB1310aH/ha for oilseed rape and ryegrass, ≥ 374.7 mL GLOB1310aH/ha for sugar beet, ≥ 655.7 mL GLOB1310aH/ha for radishes, tomato, onion and cucumber, ≥ 1147.4 mL GLOB1310aH/ha for oat and ≥ 2008.0 mL GLOB1310aH/ha barley.
No rate-response for seedling emergence and plant survival could be found for tested plant species.

NOER, ER_{25} and ER_{50} for seedling emergence and plant survival 21 DAE [mL GLOB1310aH/ha]

	Seedling emergence			Plant survival		
Test species	**NOER**	**ER_{25}**	**ER_{50}**	**NOER**	**ER_{25}**	**ER_{50}**
Błąd! Nie można odnaleźć źródła odwołania.	≥ 69.9	> 69.9	> 69.9	≥ 69.9	> 69.9	> 69.9
Błąd! Nie można odnaleźć źródła odwołania.	≥ 214.1	> 214.1	> 214.1	≥ 214.1	> 214.1	> 214.1
Błąd! Nie można odnaleźć źródła odwołania.	≥ 214.1	> 214.1	> 214.1	≥ 214.1	> 214.1	> 214.1
Błąd! Nie można odnaleźć źródła odwołania.	≥ 374.7	> 374.7	> 374.7	≥ 374.7	> 374.7	> 374.7
Błąd! Nie można odnaleźć źródła odwołania.	≥ 655.7	> 655.7	> 655.7	≥ 655.7	> 655.7	> 655.7
Błąd! Nie można odnaleźć źródła odwołania.	≥ 655.7	> 655.7	> 655.7	≥ 655.7	> 655.7	> 655.7
Błąd! Nie można odnaleźć źródła odwołania.	≥ 655.7	> 655.7	> 655.7	≥ 655.7	> 655.7	> 655.7
Błąd! Nie można odnaleźć źródła odwołania.	≥ 655.7	> 655.7	> 655.7	≥ 655.7	> 655.7	> 655.7
Błąd! Nie można odnaleźć źródła odwołania.	≥ 1147.4	> 1147.4	> 1147.4	≥ 1147.4	> 1147.4	> 1147.4
Błąd! Nie można odnaleźć źródła odwołania.	≥ 2008.0	> 2008.0	> 2008.0	≥ 2008.0	> 2008.0	> 2008.0

DAE days after 50 % emergence in the control group NOER no observed effect rate ER_X X% effect rate

Growth stage

The pre-emergence application of GLOB1310aH caused no visible effects on growth stage of lettuce, oilseed rape, sugar beet, tomato, onion, cucumber, oat and barley. The development of growth stage of ryegrass and radishes was delayed.

Effects of GLOB1310aH on growth stage 21 DAE (minimum to maximum of BBCH stage)

Test species	Application rates [mL GLOB1310aH/ha]												
	0.0	4.3	7.5	13.0	22.8	39.9	69.9	122.3	214.1	374.7	655.7	1147.4	2008.0
Błąd! Nie można odnaleźć źródła odwołania.	16-17	16-17	16-17	16-17	16-17	16-17	16-17	--	--	--	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	17	--	--	17	17	17	17	17	17	--	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	25	--	--	25	25	25	25	25	13-25	--	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	14-15	--	--	--	--	14-15	14-15	14-15	14-15	14-15	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	45	--	--	--	--	--	45	41-45	41-45	14-45	12-43	--	--
Błąd! Nie można odnaleźć źródła odwołania.	13-15	--	--	--	--	--	15	13-15	14-15	13-15	14-15	--	--
Błąd! Nie można odnaleźć źródła odwołania.	11-12	--	--	--	--	--	12	11-12	12	11-12	11-12	--	--
Błąd! Nie można odnaleźć źródła odwołania.	52	--	--	--	--	--	52	52	52	52	52	--	--
Błąd! Nie można odnaleźć źródła odwołania.	31	--	--	--	--	--	--	31	31	31	31	31	--
Błąd! Nie można odnaleźć źródła odwołania.	23-24	--	--	--	--	--	--	--	23-24	23-24	23-24	23-24	23-24

DAE days after 50 % emergence in the control group

Phytotoxicity

The pre-emergence application of GLOB1310aH caused no visible phytotoxic effects 21 DAE on lettuce, oilseed rape and tomato. Slight symptoms were seen 21 DAE in ryegrass, sugar beet, onion, cucumber, oat and barley. Severe symptoms and moribund plants detected in radishes 21 DAE.
Effects of GLOB1310aH on phytotoxicity 21 DAE [EPPO rating in %]

Test species	Application rates [mL GLOB1310aH/ha]												
	0.0	4.3	7.5	13.0	22.8	39.9	69.9	122.3	214.1	374.7	655.7	1147.4	2008.0
Błąd! Nie można odnaleźć źródła odwołania.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	--	--	--	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	0.0	0.0	0.0	0.0	0.0	0.0	--	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	0.0	0.0	0.0	0.0	0.0	12.5	--	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	0.0	0.0	0.0	0.0	10.0	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	0.0	15.7	15.7	44.3	82.9	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	0.0	0.0	0.0	0.0	0.0	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	0.0	0.0	0.0	0.0	12.5.0	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	0.0	0.0	0.0	0.0	10.0	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	--	0.0	0.0	0.0	0.0	5.0	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	--	--	0.0	0.0	7.5	15.0	17.5

DAE days after 50 % emergence in the control group

Plant height

No significant reduction of plant height could be detected for lettuce, oilseed rape, ryegrass, sugar beet, cucumber, oat and barley after pre-emergence application of GLOB1310aH. In radishes, tomato and onion the plant height was reduced significantly after pre-emergence application of GLOB1310aH.

Effects of GLOB1310aH on plant height 21 DAE [% inhibition]

Test species	Application rates [mL GLOB1310aH/ha]												
	0.0	4.3	7.5	13.0	22.8	39.9	69.9	122.3	214.1	374.7	655.7	1147.4	2008.0
Błąd! Nie można odnaleźć źródła odwołania.	0.0	-0.4	2.5	0.7	1.4	-1.8	1.4	--	--	--	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	-2.4	-5.3	-1.3	-1.8	-1.3	0.0	--	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	-0.5	2.1	-3.4	-3.1	-2.2	2.6	--	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	-2.2	-3.7	-1.2	-4.3	0.3	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	-0.7	-9.4	-1.5	5.5	54.8*	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	-1.3	0.9	3.4	11.2*	7.7*	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	0.9	8.8	4.3	7.5	16.2*	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	-25.9	-25.9	-16.8	-36.7	-33.2	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	--	-1.0	2.1	-1.6	2.5	0.4	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	--	--	1.6	-0.3	-0.9	2.3	-1.4

DAE days after 50 % emergence in the control group
statistical calculations based on plant height in cm per replicate:
* significantly different to control (Williams Multiple sequential t-test (one-sided smaller, α=0.05))

The NOER for plant height reduction for lettuce, oilseed rape, ryegrass, sugar beet, cucumber, oat and barley was higher than or equal the respective highest tested application rate of GLOB1310aH. The NOER for radishes and onion was 374.7 mL GLOB1310aH/ha and for tomato 214.1 mL GLOB1310aH/ha.
A rate-response for pant height reduction could be found for radishes with an ER_{50} of 632.9 mL GLOB1310aH/ha.

NOER, ER_{25} and ER_{50} for plant height reduction 21 DAE [mL GLOB1310aH/ha]

Test species	NOER	ER_{25}	ER_{50}
Błąd! Nie można odnaleźć źródła odwołania.	≥ 69.9	> 69.9	> 69.9
Błąd! Nie można odnaleźć źródła odwołania.	≥ 214.1	> 214.1	> 214.1
Błąd! Nie można odnaleźć źródła odwołania.	≥ 214.1	> 214.1	> 214.1
Błąd! Nie można odnaleźć źródła odwołania.	≥ 374.7	> 374.7	> 374.7
Błąd! Nie można odnaleźć źródła odwołania.	374.7	517.3	632.9
Błąd! Nie można odnaleźć źródła odwołania.	214.1	> 655.7	> 655.7
Błąd! Nie można odnaleźć źródła odwołania.	374.7	> 655.7	> 655.7
Błąd! Nie można odnaleźć źródła odwołania.	≥ 655.7	> 655.7	> 655.7
Błąd! Nie można odnaleźć źródła odwołania.	≥ 1147.4	> 1147.4	> 1147.4
Błąd! Nie można odnaleźć źródła odwołania.	≥ 2008.0	> 2008.0	> 2008.0

DAE days after 50 % emergence in the control group NOER no observed effect rate ER_X X% effect rate

Biomass

No significant reduction of biomass could be detected for lettuce, oilseed rape, ryegrass, cucumber and oat after pre-emergence application of GLOB1310aH. In sugar beet, radishes, tomato, onion and barley the biomass was reduced significantly after pre-emergence application of GLOB1310aH.

Effects of GLOB1310aH on biomass 21 DAE [% inhibition]

Test species	Application rates [mL GLOB1310aH/ha]												
	0.0	**4.3**	**7.5**	**13.0**	**22.8**	**39.9**	**69.9**	**122.3**	**214.1**	**374.7**	**655.7**	**1147.4**	**2008.0**
Błąd! Nie można odnaleźć źródła odwołania.	0.0	0.6	-0.8	-2.9	4.0	-0.8	-2.8	--	--	--	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	-3.5	-2.0	-1.0	2.9	2.3	-0.7	--	--	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	-7.0	8.8	-2.8	-4.7	-7.3	13.8	--	--	--	--
Błąd! Nie można odnaleźć źródła	0.0	--	--	--	--	3.7	9.5	2.8	-1.1	21.4*	--	--	--

odwołania.													
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	-7.6	8.3	9.7	17.3*	68.6*	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	-6.1	-0.5	2.4	6.8	16.0*	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	9.2	25.0	11.8	9.8	36.0*	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	-19.9	-16.1	-13.4	-24.2	-7.2	--	--
Błąd! Nie można odnaleźć źródła odwołania.	0.0	--	--	--	--	--	--	-8.5	5.7	-6.2	12.9	5.0	--

DAE days after 50 % emergence in the control group
statistical calculations based on biomass in g per replicate:
* significantly different to control (Williams Multiple sequential t-test (one-sided smaller, α=0.05))

The NOER for biomass reduction for lettuce, oilseed rape, ryegrass, cucumber and oat was higher than or equal the respective highest tested application rate of GLOB1310aH. The NOER for barley was 655.7 mL GLOB1310aH/ha, for tomato and onion was 374.7 mL GLOB1310aH/ha and for sugar beet and radishes 214.1 mL GLOB1310aH/ha.
A rate-response for biomass reduction could be found for radishes with an ER_{50} of 551.5 mL GLOB1310aH/ha.

NOER, ER_{25} and ER_{50} for biomass reduction 21 DAE [mL GLOB1310aH/ha]

Test species	NOER	ER_{25}	ER_{50}
Błąd! Nie można odnaleźć źródła odwołania.	≥ 69.9	> 69.9	> 69.9
Błąd! Nie można odnaleźć źródła odwołania.	≥ 214.1	> 214.1	> 214.1
Błąd! Nie można odnaleźć źródła odwołania.	≥ 214.1	> 214.1	> 214.1
Błąd! Nie można odnaleźć źródła odwołania.	214.1	> 374.7	> 374.7
Błąd! Nie można odnaleźć źródła odwołania.	214.1	402.8	551.5
Błąd! Nie można odnaleźć źródła odwołania.	374.7	> 655.7	> 655.7
Błąd! Nie można odnaleźć źródła odwołania.	374.7	n.d.	> 655.7
Błąd! Nie można odnaleźć źródła	≥ 655.7	> 655.7	> 655.7

odwołania.			
Błąd! Nie można odnaleźć źródła odwołania.	≥ 1147.4	> 1147.4	> 1147.4
Błąd! Nie można odnaleźć źródła odwołania.	655.7	> 2008.0	> 2008.0

DAE days after 50 % emergence in the control group NOER no observed effect rate ER_X X% effect rate
n.d.: not determined due to mathematical reasons or inappropriate data

Conclusion

The effects of GLOB1310aH after pre-emergence application on ten terrestrial plant species were examined at nominal application rates of 8.3, 14.4, 24.9, 43.1, 74.6, 129.1, 223.4, 386.5, 668.6 and 1156.7 mL test item/ha in 200 L water/ha under greenhouse conditions. The test endpoints for oilseed rape, cucumber, lettuce, soybean, ryegrass, great millet, sugar beet, flax, onion and oat were seedling emergence, plant survival (mortality), plant height, biomass and visual phytotoxicity 21 days after 50 % emergence in the control group (DAE).

All validity criteria of the seedling emergence and seedling growth study were met. The recoveries of the active ingredients Aclonifen and Flufenacet in the spray solution specimen of Treatment 13 were 98 % for both active ingredients. In the control specimens no active ingredient above 30 % of the LOQ was detected. Thus, the concentrations in the specimen of the biological part of the study were verified. During the study, the climate conditions were mainly kept and plants of untreated control looked healthy and grew normally. Thus, the study was valid.

No significant reduction of seedling emergence and plant survival could be detected for all tested plant species. The NOER for seedling emergence and plant survival was higher than or equal the respective highest tested application rate of GLOB1310aH.

The pre-emergence application of GLOB1310aH caused 21 DAE no visible effects on growth stage of lettuce, oilseed rape, sugar beet, tomato, onion, cucumber, oat and barley and no visible phytotoxic effects on lettuce, oilseed rape and tomato. The development of growth stage of ryegrass and radishes was delayed and slight phytotoxic symptoms were seen 21 DAE in ryegrass, sugar beet, onion, cucumber, oat and barley. Severe symptoms and moribund plants detected in radishes 21 DAE.

No significant reduction of plant height could be detected for lettuce, oilseed rape, ryegrass, sugar beet, cucumber, oat and barley. In radishes, tomato and onion the plant height was reduced significantly. The NOER for radishes and onion was 374.7 mL GLOB1310aH/ha and for tomato 214.1 mL GLOB1310aH/ha.

No significant reduction of biomass could be detected for lettuce, oilseed rape, ryegrass, cucumber and oat. In sugar beet, radishes, tomato, onion and barley the biomass was reduced significantly. The NOER for barley was 655.7 mL GLOB1310aH/ha, for tomato and onion 374.7 mL GLOB1310aH/ha and for sugar beet and radishes 214.1 mL GLOB1310aH/ha.

A rate-response for plant height reduction and for biomass reduction could be found for radishes with an $ER_{50\ plant\ height}$ of 632.9 mL GLOB1310aH/ha and an $ER_{50\ biomass}$ of 551.5 mL GLOB1310aH/ha.

A 2.6.3 KCP 10.6.3 Extended laboratory studies on non-target plants

No new studies are submitted in the frame of this application.

A 2.7 KCP 10.7 Effects on other terrestrial organisms (flora and fauna)

No new studies are submitted in the frame of this application.

A 2.8 KCP 10.8 Monitoring data

No new studies are submitted in the frame of this application.